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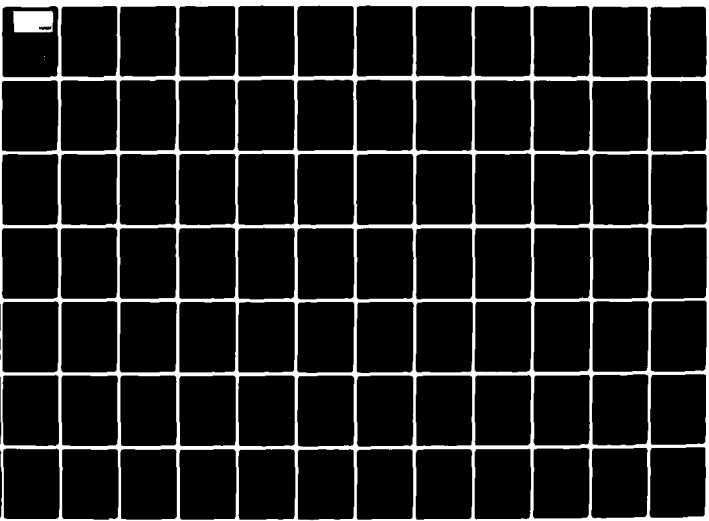
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REPORT

MRI Project No. 4274-B

SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM
OF 2,4,6-TRINITROTOLUENE AS A FUNCTION
OF ROUTE OF ADMINISTRATION

FINAL REPORT

A. Monaem El-hawari
John R. Hodgson
J. Mark Winston
Mary D. Sawyer
Maxine Hainje
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June 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

Contract No. DAMD17-76-C-6066

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

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Species Differences	Tissue Distribution	Intratracheal	Rabbit
Disposition	Excretion	Mouse	
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The disposition and metabolism of 2,4,6-trinitrotoluene (TNT) was studied in rats, mice, rabbits, and dogs following oral, dermal or intratracheal administration of single doses of ¹⁴ C-ring labeled compound. The objective was to determine possible species and sex differences as a function of route of administration as a rationale for the design of chronic studies.			

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20. Abstract (continued)

TNT was absorbed in all species by all routes of administration with the most extensive absorption occurring after intratracheal instillation. Dermal absorption was highest in rabbits followed by mice, rats, and dogs. Species differences in the rate of oral absorption could not be accurately assessed. Excretion was primarily in urine and to a lesser extent in feces. Extensive biliary excretion was also noted. Blood and tissue levels in females were generally higher than in males.

TNT was extensively metabolized in all species; radioactivity excreted in urine primarily as the glucuronide conjugates. Most metabolites were reduction products including the 2- and 4-hydroxylamine and 2- and 4-monoaminodinitro and 2,6- and 4,6-diaminomono-nitro derivatives. Trace quantities of TNT, trinitrobenzyl alcohol and trinitrobenzoic acid were detected occasionally.

Urinary metabolic profiles were similar qualitatively in mice, rats, and dogs; profiles in rabbits were dissimilar. Also, metabolic profiles demonstrated after intratracheal instillation differed significantly from those obtained after oral or dermal administration.

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PREFACE

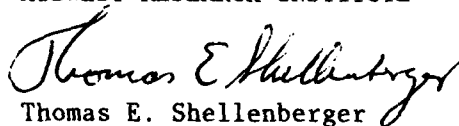
This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-76-C-6066 entitled "Evaluation of Difference in Mammalian Metabolism of Trinitrotoluene (TNT) as a Function of Route of Administration and Carcinogen Testing." The work was supported by the U.S. Army Medical Research and Development Command, Department of the Army. Cpt. Ronald N. Shiotsuka, MSC, Environmental Protection Research Division, U.S. Army Medical Bioengineering Research and Development Laboratory, was the Contract Officer's technical representative.

The work was conducted in the Biological Science Division under the direction of Dr. William B. House, between June 29, 1976 and March 31, 1978, and Dr. Harold M. Hubbard, between April 1 and November 30, 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Deputy Director, with Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology, and Dr. A. Monaem El-hawari, Senior Toxicologist, as the successive Principal Investigators. Dr. J. Mark Winston performed and supervised the inhalation investigations. Ms. Mary D. Sawyer and Ms. M. Hainje, Junior Biologists, performed the animal experiments, radioactivity and TLC analysis. Mr. W. B. Butron, Associate Chemist, synthesized the potential metabolites. Dr. E. Murrill, Senior Advisor for Chemistry, supervised the HPLC analysis.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

Approved for:

MIDWEST RESEARCH INSTITUTE



Thomas E. Shellenberger
Director
Toxicology Department

June 1981

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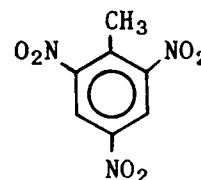
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SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM
OF 2,4,6-TRINITROTOLUENE AS A FUNCTION
OF ROUTE OF ADMINISTRATION

EXECUTIVE SUMMARY

The disposition (absorption, tissue distribution, and excretion) and metabolism of 2,4,6-trinitrotoluene (TNT, I) were studied in rats, mice, rabbits, and dogs after oral, dermal, or intratracheal administration of single doses of the ring-¹⁴C-labeled compound. The primary objective of these studies was to determine the species differences, if any, in the metabolic fate of TNT as a function of route of administration for possible use as a rationale for selecting an appropriate species, sex, and route of exposure for subsequent chronic studies. Specifically, the intent was to evaluate the metabolic behavior of TNT after oral, inhalation, and dermal exposures in order to establish if oral exposure could be used in lieu of other routes in any subsequent carcinogenicity studies. Since TNT aerosols prepared using methods reported herein were not adequate for inhalation exposure, the intratracheal instillation method was used in an attempt to simulate pulmonary absorption of the test chemical.



(I) TNT

TNT administered orally to rats, mice, rabbits, and dogs was readily absorbed and excreted mainly in urine and to a lesser extent in feces. Major portions of the administered doses were recovered in the GI tracts (Table A). Urine of rats and mice, but not of rabbits and dogs, was bright red in color. The extent of absorption could not be accurately assessed from these studies since radioactivity recovered in the feces and GI tracts represents a balance between absorption, biliary excretion, and intestinal reabsorption. At 24 hr, blood and tissue of dogs contained higher radioactivity (percent of dose) than did blood and tissue of rats, mice, and rabbits. Generally, higher ¹⁴C levels were recovered in blood and tissue of female animals. Blood, liver, kidneys, and occasionally spleen and lungs contained high levels of radioactivity; rabbit lung tissue contained 9 to 14 times higher ¹⁴C levels than did blood. Other tissues, including brain and muscle, contained detectable levels of radioactivity.

TABLE A

RECOVERY OF RADIOACTIVITY (PERCENT OF DOSE) AT 24 HR
AFTER ORAL ADMINISTRATION OF ^{14}C -TNT

	Rats		Mice		Rabbits		Dogs	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine	52.72	64.55	41.91	42.87	66.30	78.86	55.92	60.16
Feces	8.05	2.06	22.01	8.96	1.78	1.83	5.41	16.80
GI Tract	29.76	33.94	13.45	7.42	7.50	4.72	10.00	4.40
Blood	0.20	0.29	0.90	0.07	0.28	0.44	1.38	1.96
Tissue	0.89	1.59	2.18	1.11	1.80	3.10	4.64	4.96
Recovery	91.62	102.43	80.06	60.44	77.65	88.94	77.35	88.26

Following dermal application, TNT was absorbed by the four species studied. Absorption was highest in rabbits followed by mice, rats, and dogs (Table B). Most of the TNT absorbed was eliminated in urine. Radioactivity was also recovered in the feces and GI tracts indicating probable excretion via bile. Total urinary and fecal excretion at 24 hr following dermal application was less than after oral administration of the same dose. As with the oral dosing, urine of dermally dosed rats and mice was bright red. Residual radioactivity was higher in fat of all species following dermal application than after oral dosing. Radioactivity was also highly concentrated in residual bile and liver after both dermal and oral exposure. In rabbits and dogs, absorption and excretion of TNT appeared similar at both dose levels studied (5 and 50 mg/kg) although in dogs, blood content (percent of dose) was higher after the high dose of TNT.

TABLE B

RECOVERY OF RADIOACTIVITY (PERCENT OF DOSE) AT 24 HR
AFTER ORAL OR DERMAL TREATMENT WITH ^{14}C -TNT

	Rats		Mice		Rabbits		Dogs	
	Oral	Dermal	Oral	Dermal	Oral	Dermal	Oral	Dermal
Urine	59.54	17.35	59.05	22.68	68.07	52.85	70.50	11.73
Feces	10.72	1.32	24.07	14.17	5.45	7.80	9.00	1.71
GI Tract	20.24	3.11	10.19	3.61	19.74	5.76	14.63	1.68
Blood	0.25	0.23	0.17	0.17	0.40	0.26	1.11	0.26
Tissue	1.44	0.68	0.91	1.04	1.91	1.59	4.15	1.42
Recovery	92.19	22.76	94.39	41.69	95.57	68.26	99.39	16.81

Extensive absorption was demonstrated when a suspension of ^{14}C -TNT was instilled intratracheally into rats. Radioactivity appeared in the blood quickly and decreased slowly during a 4-hr period. Blood ^{14}C levels were higher and the urinary excretion levels were greater in these rats than in rats treated orally under the same conditions (Table C). In bile duct-cannulated rats, large amounts of radioactivity were excreted in the bile, urine, and the GI tract. Urinary and biliary excretion rates were also higher in these rats than in rats treated orally. Enterohepatic circulation of TNT and its metabolites (excretion in bile followed by absorption and reexcretion in urine or feces) seemed to occur. The urine of rats from both routes of administration was bright red. At 4 hr, residual radioactivity in most tissues was higher after intratracheal instillation than after oral administration. After both routes of administration, fat contained the highest content of radioactivity, and lung tissue had higher ^{14}C concentrations than did blood or liver. Levels of ^{14}C in blood and tissues of female rats were about two times higher than in the males.

TABLE C

RECOVERY OF RADIOACTIVITY (PERCENT OF DOSE) AT 4 HR AFTER
ORAL OR INTRATRACHEAL ADMINISTRATION OF ^{14}C -TNT

	Intact Rats				Bile Duct-Cannulated Rats			
	Oral		Intratracheal		Oral		Intratracheal	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine	14.63	10.01	19.32	13.23	10.73	8.42	17.50	12.68
Bile	-	-	-	-	11.57	9.67	19.75	14.51
GI Tract	73.70	79.02	18.24	12.06	68.29	64.22	1.79	2.92
Blood	1.34	2.78	2.24	4.29	1.34	2.78	2.24	4.29
Tissue	3.60	6.12	5.80	10.58	3.60	6.12	5.80	10.58
Recovery	93.27	97.93	45.60	40.16	95.53	91.21	47.06	44.98

Because of the presence of four functional groups on the TNT molecule, a variety of metabolites resulting from oxidation, reduction, and conjugation could be formed. Simultaneous oxidation and reduction followed by conjugation is also a possibility. Most TNT metabolic products in urine and bile are highly polar with very low extractability in organic solvents (ether and ethyl acetate). Mild acidification (dilute HCl) before ethyl acetate extraction proved essential for increased recovery. A method was developed for the fractionation of the radioactive urinary metabolites into subgroups according to their solubilities in ether under different pH conditions. Metabolites were separated by thin-layer chromatography (TLC). The use of gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC) was discontinued after it was apparent that neither technique offered added advantages in the separation of TNT metabolites. Tentative identification of metabolites was carried out by comparing solubility characteristics, reactions with specific spraying reagents, and R_f values of the metabolites with those of standard reference compounds.

TNT was metabolized extensively in all species examined, whether treatment was oral, dermal, or intratracheal. Large portions of the products were conjugated with glucuronic acid, but no conjugation with sulfuric acid was detected. Other conjugates or inorganic salts of TNT metabolites were probably present. Most of the metabolic products were reduction derivatives, including the 2- and 4-hydroxylamines, the 2- and 4-monoaminodinitro and the 2,6- and 4,6-diaminomononitro derivatives. The trinitrobenzyl alcohol and the trinitrobenzoic acid seemed to be present, but confirmation was not possible. The parent compound, TNT, was demonstrated in the urine of some species in only minute quantities. The mild extraction procedures used minimized the alterations of the hydroxylamines to the azoxytoluene, but some of the latter was present, especially after fractionation of the urinary products in the presence of NaOH. Other products of TNT metabolism remain unidentified.

The metabolic profiles of urine from rats, mice, and dogs differed only quantitatively. Urine of rats contained larger amounts of the 4,6-diamine and, to a lesser extent, the 2,6-diamine and either or both of the 2- or 6-monoamines. The 2- and 4-hydroxylamines and some azoxytoluene (probably formed during fractionation) were present in small quantities. The presence of appreciable amounts of the trinitrobenzyl alcohol and trinitrobenzoic acid was suggested by comparison with authentic samples. Metabolic profiles of urine from male and female rats showed no significant differences. The amounts of glucuronides in urine collected from bile duct-cannulated rats were lower than those collected from noncannulated rats. In addition, the 4-hr urine contained more of the polar metabolites and more parent TNT.

Compared to rat urine, mouse urine contained smaller quantities of the polar metabolites and the diamines and more of the monoamines and hydroxylamines. Mouse urine also contained considerable amounts of the trinitrobenzyl alcohol and trinitrobenzoic acid. The metabolic profiles of dog urine contained appreciable amounts of diamines and monoamines and probably the trinitrobenzyl alcohol and the trinitrobenzoic acid. Only traces of the 4-hydroxylamine, the 2-hydroxylamine, and some azoxytoluene (which seemed to be formed during fractionation) were present. Rabbit urine showed an unique profile which differed quantitatively, and probably qualitatively, from that of rats, mice, and dogs. The presence of larger quantities of monoamines and hydroxylamines was demonstrated. In addition, it contained either or both of the diamines, trinitrobenzyl alcohol, and trinitrobenzoic acid. TNT and the azoxytoluene were absent from fresh urine, but some of the latter was formed during fractionation in the presence of NaOH.

Major quantitative differences were demonstrated in the urinary metabolic profiles of orally versus intratracheally treated rats. On the other hand, the differences between urine profiles obtained from orally and dermally treated animals were minimal, although larger amounts of TNT were eliminated after dermal application. The extractable radioactivity increased considerably after β -glucuronidase hydrolysis of urine from different species following different routes of administration. However, major changes in the metabolic profiles were not apparent.

I. INTRODUCTION

Under Contract No. DAMD-17-76-C-6066, entitled "Evaluation of Differences in Mammalian Metabolism of Trinitrotoluene (TNT) as a Function of Route of Administration and Carcinogenic Testing," MRI conducted experimental studies to achieve the following objectives:

1. Develop a suitable method for the generation of an aerosol of TNT in sufficient concentrations for metabolic studies.
2. Determine the disposition (absorption, tissue distribution and excretion) of TNT in four animal species (rat, mouse, rabbit, and dog) after oral, dermal, and intratracheal administration.
3. Develop suitable methods for the characterization of the metabolic products of TNT in the urine of these species.

The primary objective of these studies was to develop a data base for selecting an appropriate animal model for subsequent chronic studies and to determine whether oral administration could be used as an alternative to other (e.g., dermal and inhalation) routes in any future carcinogenicity studies.

The initial approach was to compare the absorption, distribution, metabolism, and elimination of TNT in several species following oral and inhalation exposures. Initial efforts were directed to procurement or synthesis of some potential metabolites, development of methods to separate and identify these metabolites, the conduct of oral dosing studies, and the evaluation of methods to produce aerosols applicable for inhalation exposures. Efforts to generate satisfactory TNT aerosols in concentrations which are suitable for metabolic studies were not successful; and following discussions with the project officer, intratracheal instillation to simulate inhalation exposure was substituted. Dermal exposure studies were subsequently incorporated into the project. The research efforts thereafter were directed to disposition and metabolic studies following oral, dermal, or intratracheal administration of TNT using rats, mice, rabbits, and dogs.

II. BACKGROUND

A. Production and Use

2,4,6-Trinitrotoluene (TNT) was first synthesized by Wilbrand in 1863, but it was not prepared on an industrial scale until 1891. A few years later, it found wide application as an explosive for shells, bombs, and grenades.¹ Millions of tons of TNT were produced during World Wars I and II. In 1973, an estimated 200,000 tons were manufactured in the U.S. Army ammunition plants.² TNT is the most widely used military explosive because of its low melting point, comparative safety during manufacture, and stability during transportation and storage.^{3,4} It is also used as an intermediate in the synthesis of dyes and photographic chemicals.

B. Human Toxicity

The manufacture of TNT creates fumes of TNT and other decomposition products. Some workers exposed to TNT by breathing the fumes or by skin contact have experienced harmful effects, including liver malfunction⁵⁻⁷ and decreased ability of the bone marrow to produce blood cells.⁸⁻¹² TNT also damages the heart,¹³ blood vessels,¹⁴ kidney,^{15,16} and pancreas,¹⁷ and probably causes cataracts.¹⁸⁻²³ Exposure to TNT decreases the oxygen-carrying capability of the red blood cells due to formation of methemoglobin²⁴ and nitric oxide hemoglobin.²⁵ Hemolytic anemia has been reported in TNT workers deficient in glucose-6-phosphate dehydrogenase.^{26,27} Persons poisoned with TNT have urine that is red but not bloody.²⁸ Deaths have been mainly attributed to jaundice, aplastic anemia, or both.^{5,6} To date, no carcinogenic effect has been reported among munitions workers exposed to TNT.²⁹

During World War I, a large number of cases of toxic jaundice were reported among TNT workers in the United States and Europe, many of which ended in fatality.³⁰⁻³³ The implementation of strict hygiene practices during World War II resulted in a dramatic decrease in the number of fatalities. Currently, the Occupational Safety and Health Administration limits exposure to TNT to 1.5 mg/m³ in air (8-hr time-weighted average). To provide greater protection to munition workers, the U.S. Army has lowered its acceptable TNT exposure levels to 0.5 mg/m³ over the same time period.

C. Animal Toxicity

Liver and blood diseases have appeared in experimental animals exposed to TNT.³⁴⁻³⁹ No pulmonary lesions or lung neoplastic effects have been demonstrated in guinea pigs, rats, or mice exposed to TNT.²⁹ However, after 6 months of topical application of TNT to Wistar rats, bone marrow cells exhibited chromatid changes, chromosomal breaks, and dislocations, but no change in chromosomal numbers.⁴⁰ Studies on TNT mutagenicity using histidine-requiring strains of *Salmonella typhimurium* (Ames test system) have indicated that TNT is mutagenic. However, the major microbial metabolites of TNT appear to be nonmutagenic.⁴¹

Experimental animals differ in susceptibility to TNT toxicity. Cats are more sensitive than rats, rabbits, dogs, and monkeys. It has been suggested that these differences are due, at least in part, to differences in the fate of TNT in these species.⁴² It has also been shown that various microorganisms biodegrade TNT; *Escherichia coli* can reduce the nitro groups to the respective amines.⁴³⁻⁴⁵ Degradation of TNT by bacteria and sunlight gives TNT wastewater a pink or red color.

D. Absorption

TNT may enter the body through the gastrointestinal tract, the skin, or the lungs.⁴⁶⁻⁴⁸ It is believed that the skin is the chief route of absorption.⁴⁶ Voegtlin et al.⁴⁷ have demonstrated that in humans, skin absorption takes place readily through the hands, neck, and face; oily skin and sweat favors absorption.⁴⁹ Although some experiments have demonstrated that TNT is absorbed when introduced as dust in the lower air passages, Putnam and Herman⁴⁶ suggested that intoxication via the respiratory tract rarely, if ever, occurs.

During exposure to TNT, the powder may be ingested by mouth and gain access to the stomach. TNT workers have complained about a bitter taste in their mouth. When two human subjects received daily doses of TNT for four successive days, a portion of the TNT administered was recovered from the urine in the form of the reduced metabolite, 2,6-dinitro-4-aminotoluene.⁵⁰ Experimentally, guinea pigs fed oral doses of TNT with milk developed diarrhea, and poisoning symptoms were apparent for 3 to 14 days.⁵¹

TNT was absorbed through the skin of swine as indicated by the presence of the reduced metabolite, 2,6-dinitro-4-aminotoluene in urine.⁵² Also, Haythorn⁵³ reported that guinea pigs and rabbits rubbed repeatedly with 10% TNT in lanolin showed liver lesions and a positive Webster's test (a test introduced in 1916 by Webster which has been used to detect TNT metabolites in human urine in cases of intoxication). However, when Haythorn rubbed TNT powder on his arm for several consecutive days, he could not demonstrate a positive Webster's test and did not feel any ill effects.⁵³ In another study, TNT was rubbed into the palms of two human subjects and kept under rubber gloves for 8 hr.⁵⁴ Traces of the metabolite 2,6-dinitro-4-aminotoluene were found in the urine collected during and after the exposure to TNT.

Absorption through the respiratory system has also been examined by Haythorn.⁵³ When guinea pigs were exposed to fumes of volatilized TNT for 30 days, no lesions ascribed to TNT were observed, but the animals died from the heat used to volatilize TNT. In another series of experiments, TNT powder was introduced into the lungs of experimental animals, but no toxicity developed. This led Haythorn to conclude that the lung is unimportant as a route of intoxication from TNT. Later, however, Von Oettingen et al., demonstrated that 75% of a TNT dose administered to dogs by insufflation was absorbed from the respiratory tract.⁴⁸

E. Retention and Excretion

Voegtlin et al. believed that TNT is retained in the body for a considerable period of time, as indicated by the progressive anemia after single doses of TNT and by the slow recovery of the animals.⁴⁷ However, when Von Oettingen and his co-workers administered TNT to dogs by insufflation for a period of 17 weeks, significant amounts of TNT or its metabolite, 2,6-dinitro-4-aminotoluene, were not found in any organ or tissue examined at the end of the study.⁴⁸ These authors concluded that TNT is not retained to any considerable extent in these organs. However, conclusions from these early studies regarding storage or excretion of TNT and its metabolites are hampered by the insensitivity of the methods used to examine TNT or the reduced metabolites, aminotoluenes.

Earlier studies suggested that the urine was the main route of excretion for TNT. In rats, 20% of a single oral dose of TNT was excreted in the urine as diazotizable aromatic amino compounds.⁵⁵ Human volunteers excreted an average of 40% of small oral doses of TNT as aromatic amino compounds in the urine. In other experiments, humans receiving TNT excreted about 3% of the ingested dose as 4-amino and 2,4-diamino products; concentration of these metabolites fell almost to zero within 24 hr after the last dose.⁵⁰ Although it was suggested that TNT was excreted in bile,⁴⁷ Haythorn could not obtain a positive Webster's test in the feces of animals given TNT by any route except orally.⁵³

F. Metabolism

Since the beginning of this century, extensive work has been carried out to isolate and identify TNT metabolites in animals⁵⁶⁻⁵⁸ and humans.^{55,57} Only limited success has been achieved because of the difficulties encountered during the isolation procedures. Low recovery was encountered when urine samples were extracted with ether. Even after acidification of urine, no more than 15% of the administered doses were recovered in ether. The use of strong acid or base should be avoided since this undoubtedly causes alterations of the metabolites during the extraction process. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene, which was reported as one of the TNT metabolites in rabbit and human urine,⁵⁷ was found later to be an artifact that was formed from the 4-hydroxylamine under the conditions of the isolation procedure. This azoxytoluene was shown to be absent from freshly voided urine of rabbits given TNT.⁵⁶

TNT metabolism conceivably may involve alterations of the four functional groups, the open positions on the benzene ring, or scission of this ring. Ring cleavage rarely occurs and probably plays little, if any, role in the metabolism of TNT. However, a variety of other metabolic products could be formed. These may result from oxidation of the methyl group to alcohol, aldehyde, or acid; oxidation of the benzene nucleus to phenols; reduction of one or more of the nitro groups to hydroxylamino or amino compounds with the possibility of coupling of some of these metabolites; and conjugation of one or more of the resulting products (alcohols, acids, amines, hydroxylamines, etc.) to yield glucuronides, ethereal sulfates,

substituted hippuric acid, or glutathione conjugates. Simultaneous oxidation and reduction followed by conjugation is also a possibility. These hypothetical pathways, which are shown in Figure 1, illustrate the complexity of the metabolism of TNT. The problem of metabolite identification is complicated by the similar solubility characteristics possessed by these compounds of such closely related chemical structure.

Earlier studies have shown that the reduction products, 4-amino-2,6-dinitrotoluene and 2,6,2',6'-tetranitro-4,4'-azoxytoluene, are excreted in the urine of workers exposed to TNT.^{42,57} Reduction of a single nitro group of TNT was also shown to occur in experimental animals leading to the formation of 4-amino- and 6-amino-dinitrotoluenes.⁵⁶ Channon et al.⁵⁶ postulated that the first step in the reduction of the nitro group is the production of a hydroxylamine derivative. The 4-hydroxylamino-2,6-dinitrotoluene was isolated as an aldoxime after reaction with benzaldehyde; the isomer 2-hydroxylamino-4,6-dinitrotoluene was not isolated, but the isolation of the reduction product 2-amino-4,6-dinitrotoluene led to the conclusion that the 2-hydroxylamine is a step in its formation.

The isolation of hydroxylamine is of interest since Wyon found the hydroxylamine derivatives to be more toxic than the parent TNT.⁵⁹ The hydroxylamine is a powerful methemoglobin producer *in vitro*, while TNT itself is only a weak producer of methemoglobin.⁵⁹ In addition, the formation of a hydroxylamine is implicated in the carcinogenicity responses induced by several carcinogenic amino and nitro compounds.⁶⁰ Only 1% of the TNT dose was accounted for as hydroxylamine.⁵⁶ This, however, seems to be less than the actual amount present because of the extreme ease of conversion to the azoxy derivative.

Oxidation of the methyl group of TNT may result in the formation of alcohol or acid. These oxidation processes are hypothetical and are based on some indirect evidence obtained from the studies of Channon et al.⁵⁶ Rabbits excreted some TNT metabolites as glucuronides, which were believed to arise from oxidation products of TNT such as trinitrobenzyl alcohol. However, the possibility of glucuronide conjugation with the amino or hydroxylamino derivatives was not considered. The suggestion that nitrophenylenediamine is excreted in rat urine also indicates that this oxidative pathway may be operative.⁵⁵ The loss of the methyl group could probably occur by oxidation of TNT to the alcohol, then the acid, followed by decarboxylation and reduction of the nitro group.⁶¹ Aminonitroresol is another oxidation product whose presence in rat urine was suggested. The mechanism of its formation is not known.

Early studies have suggested that urine from TNT workers contained the same metabolites reported in rabbit urine, namely 4-hydroxylamino-2,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and 2-amino-4,6-dinitrotoluene.⁵⁶ Rat urine contained, in addition to the monoamines, 2,4-diamino-6-nitrotoluene and probably 5-nitrophenylenediamine.⁵⁵ On the other hand, Snyder⁵⁸ was unable to demonstrate the presence of TNT, its oxidation products (alcohol, aldehyde, or acid), or its reduction products (diamino- and triaminotoluenes) in the urine of dogs which received TNT orally.

Glucuronide conjugation appears to play an important role in the metabolism of TNT. Other conjugates and probably inorganic salts may also be formed. Channon et al.⁵⁶ found that, even after acidification of rabbit urine, no more than 15% of the administered dose was excreted as compounds soluble in ether. The ether extracts contained metabolites excreted in an unconjugated form and possibly small amounts of acetylated amino derivatives. The remainder of the doses administered were probably eliminated as conjugates, e.g., glucuronides and sulfates. The excretion of compounds in combination with glucuronic acid was suggested based on an increase in glucuronides in urine after TNT dosing.

In vitro experiments suggested that the liver is a major site for TNT biotransformation.⁶² Studies using liver, muscle, and heart preparations showed that TNT was reduced by liver homogenates to 4-amino-2,6-dinitrotoluene. The rate of reduction was more rapid under anaerobic conditions. TNT metabolism occurred in a system containing reduced nicotinamide dinucleotide (NADH) and a purified flavoprotein. It was also suggested that TNT was reduced to hydroxylamines by xanthine oxidase.

III. MATERIALS

A. Animals

The adult male and female animals used in these studies were obtained from commercial suppliers. Swiss albino CD₁® mice (20 to 30 g) and Sprague-Dawley CD® rats (200 to 300 g) were purchased from Charles River Breeding Laboratories (North Wilmington, Massachusetts). New Zealand rabbits (3 to 4 kg) were purchased from Small Stock Industries (Pea Ridge, Arkansas). Beagle dogs (8 to 14 kg) were purchased from Hazleton Research Animals (Cumberland, Virginia). All animals were kept for at least 1 week prior to dosing in temperature-controlled (74 ± 2°F) and humidity-controlled (40 to 60%) rooms maintained on a 12-hr light and dark cycle. Diet consisted of commercially available rodent, rabbit, and dog chow.

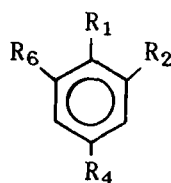
B. Chemicals

1. Test compound: Military grade 2,4,6-trinitrotoluene (TNT) was supplied by Mr. Ralph Hauze of the Volunteer Army Ammunition Plant (Chattanooga, Tennessee). Gas-liquid chromatography (GLC) analyses indicated that the test compound contained 99.82% TNT and 0.18% 2,4-dinitrotoluene (DNT).

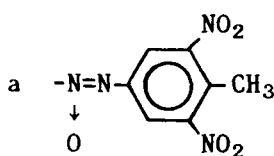
Radiolabeled TNT-(ring-UL-¹⁴C) with specific activity of 19.76 µCi/mg was purchased from Pathfinder Laboratories (St. Louis, Missouri). The radiochemical purity was found to be greater than 98% as determined by thin-layer chromatography (TLC).

2. Reference standards: Reference standards of some potential TNT metabolites were purchased from K&K Laboratories (Plainview, New York), synthesized in MRI laboratories, or obtained from Dr. N. E. Burlinson of Naval Surface Weapons Center (NSWC, White Oak, Silver Spring, Maryland). These standards are shown below.

The dinitrohydroxylaminotoluenes were prepared by reduction of TNT with a mixture of ammonium hydroxide and hydrogen sulfide according to the method of Elvove.⁶³ Examination of the product by TLC revealed a mixture of two major products. Attempts to separate these products by gravity column chromatography, using silica gel with various solvents, were unsuccessful. The mixture was resolved by the use of high performance liquid chromatography (HPLC) on a Woelm silica gel column. Elution with 25% petroleum ether in methylene chloride gave a yellow solid, m.p. 169-170°C. TLC on silica gel G using chloroform as the developing solvent gave a single spot with an R_f of 0.7. Further elution with methylene chloride gave a second solid, m.p. 148-149°C (R_f = 0.5 in the same system). Spectral analysis of the two products by nuclear magnetic resonance confirmed that both were dinitrohydroxylaminotoluenes, but the location of the hydroxylamine group in each was uncertain. TLC comparison with authentic samples of the two hydroxylamines obtained from Dr. N. E. Burlinson of NSWC indicated that the product with m.p. 148-149°C was 2,6-dinitro-4-hydroxylaminotoluene and the compound with m.p. 169-170°C was 2,4-dinitro-6-hydroxylaminotoluene.



<u>Standard</u>	<u>R₁</u>	<u>R₂</u>	<u>R₄</u>	<u>R₆</u>
1. Trinitrotoluene (TNT)	CH ₃	NO ₂	NO ₂	NO ₂
2. Trinitrobenzyl alcohol	CH ₂ OH	NO ₂	NO ₂	NO ₂
3. Trinitrobenzoic acid	COOH	NO ₂	NO ₂	NO ₂
4. 1-Amino-2,6-dinitrotoluene	CH ₃	NO ₂	NH ₂	NO ₂
5. 2-Amino-4,6-dinitrotoluene	CH ₃	NH ₂	NO ₂	NO ₂
6. 4,6-Diamino-2-nitrotoluene	CH ₃	NO ₂	NH ₂	NH ₂
7. 2,6-Diamino-4-nitrotoluene	CH ₃	NH ₂	NO ₂	NH ₂
8. 4-Hydroxylamino-2,6-dinitrotoluene	CH ₃	NO ₂	NHOH	NO ₂
9. 2-Hydroxylamino-4,6-dinitrotoluene	CH ₃	NHOH	NO ₂	NO ₂
10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene	CH ₃	NO ₂	a	NO ₂



Attempts to prepare 2,4,6-trinitrobenzyl alcohol by reducing the corresponding acid with boranemethyl sulfide gave a mixture of four products. Therefore, a modification of a procedure by Ganguly⁶⁴ was used. It involved treatment of TNT with sodium hypobromite to give the trinitrobenzyl bromide, followed by hydrolysis to the alcohol. Infrared and TLC analyses indicated that the alcohol was identical to a reference sample of 2,4,6-trinitrobenzyl alcohol obtained from NSWC.

Attempts to synthesize 4-amino-2,6-dinitrobenzyl alcohol by selectively reducing the 4-nitro group of the 2,4,6-trinitrobenzyl alcohol using ammonium hydroxide-hydrogen sulfide resulted in a mixture of products; attempts to purify this mixture were unsuccessful. In another experiment, the alcohol was reduced with hydrogen sulfide in dioxane.⁶⁵ This also produced a mixture of three products which proved difficult to purify.

IV. AEROSOL PRODUCTION

A. Particle Size Reduction

1. Ball milling: The first approach to reducing TNT to "respirable" size particles was to utilize conventional ball milling techniques. Approximately 200 g of military grade TNT was placed in a ball mill jar with 28 to 32 porcelain balls. The TNT was milled for varying lengths of time up to 2 hr, and samples were removed at different intervals for microscopic determination of particle size. The minimum particle size range achieved was 20 to 28 μm after 30 min of ball milling. Ball milling for longer periods of time did not result in a further reduction in particle size. After ball milling, the TNT had a tendency to clump together in the jar and adhere to the sides of the mill jar.

2. Nebulization of TNT: Nebulization of TNT from a TNT-acetone solution was attempted to produce respirable size TNT particles. This method utilized the FK-8 nebulizer gun developed at Edgewood Arsenal. The FK-8 gun is designed to produce an aerosol at the rate of 2 ml/30 sec or 240 ml/hr with a particle size of 1 to 2 μm . For the TNT experiments, nitrogen at a pressure of 60 to 70 psi was used to produce the TNT particles from a TNT-acetone solution. For these studies, the aerosol from the FK-8 gun was directed into a 5-gal. widemouthed glass jar.

Using the FK-8 nebulizer gun, it was possible to produce TNT particles in the 1- to 2- μm size range as determined from microscopic examination. However, the TNT particles produced had the tendency to adhere to the sides of the glass container. In addition, it appeared that the separation of the TNT particles from the acetone would pose a significant problem in producing an aerosol suitable for the exposure of animals.

3. Aspirator method: A TNT-acetone solution was dispersed through a cold water aspirator vortex. The TNT particles were precipitated in the water and filtered. The filtered particles were then washed with 95% ethanol followed by an ether wash to obtain dry particles. The dry TNT particles were then sized by light microscopy and were found primarily to be greater than 20 μm in size. Therefore, the aspirator method was not suitable for producing TNT particles of respirable size.

4. Ball milling in a cold atmosphere: As discussed above, conventional ball milling techniques produced TNT particles in the 20- to 28- μm size range. It was felt that the softness of TNT (a hardness of 1.4 on the Mohs scale) might have contributed substantially to the failure to produce smaller particles by ball milling. By hardening the TNT particles, it was anticipated that ball milling might result in a further reduction in TNT particles. To harden the TNT particles, ball milling in a cold atmosphere was attempted.

The initial approach to producing a cold atmosphere was to add dry ice to the ball mill jar. Although the addition of dry ice in the TNT reduced the temperature of the jar initially, the ball milling procedure

quickly dissipated the dry ice and the jar rapidly returned to ambient temperature. In addition, adding the dry ice to the jar resulted in a problem with moisture collection which in turn appeared to aggravate the clumping problem. The particle size range produced by this method was essentially the same as that obtained with conventional ball milling, 20 to 28 μm .

A second approach involved adding liquid nitrogen to the TNT. Upon addition of the liquid nitrogen to the mill jar, the TNT powder present froze into a solid sheet. After 20 min of ball milling, the particles examined microscopically were greater than 15 μm in size. As with the dry ice method, the liquid nitrogen quickly dissipated, resulting in a rapid return to ambient temperature.

To keep the ball mill jar at a reduced temperature throughout ball milling, the jar was immersed in a dry ice-acetone bath. Using this approach, it was possible to maintain the ball mill jar at a reduced temperature throughout the milling procedure. After ball milling for 1 hr in the dry ice-acetone bath, the particles obtained were greater than 10 μm in size. Ball milling in the dry ice-acetone bath for additional lengths of time did not further reduce the TNT particle size.

5. Jet pulverizer system: The jet pulverizer system (Jet Pulverizer Company, Palmyra, New Jersey) was attempted to reduce TNT to the 1- μm particle size. The jet pulverizer is a fluid energy mill in which the fluid energy (in this case nitrogen gas) is admitted in fine, high velocity streams around the periphery of a grinding and classifying chamber. The high order of turbulence created causes the particles to grind upon themselves and be ruptured, forming smaller particles.

After determining that substances of the softness of TNT could be ground successfully with the jet pulverizer system, experiments were undertaken to reduce the TNT to the desired particle size. The TNT was fed to the pulverizer using a vibrating trough which in turn was fed by a vibrating feed hopper in order to maintain a constant feed of material to the pulverizer.

It was found that TNT particles 1 to 3 μm in size could be produced using the jet pulverizer. Microscopic examination of these particles showed the particles to be spherical and 1 to 3 μm in diameter. The particles existed as both single particles and agglomerates of the 1- to 3- μm particles. After setting for a number of days, the TNT particles again displayed the tendency to clump together, although the individual particles remained 1 to 3 μm in size. Therefore, it appears the jet pulverizer is suitable for reducing TNT to a 1- to 3- μm particle size.

B. Aerosol Generation

1. Generation from jet pulverizer-produced particles: After obtaining respirable (1 to 3 μm) TNT particles with the jet pulverizer, attempts were made to produce a suitable aerosol for animal studies. Pilot

studies of aerosol generation centered on two methods. The first method involved the nebulizing of TNT suspensions. The TNT was suspended in either Tween-80 or propylene glycol. For these pilot studies, a 5-gal. widemouthed glass jar was used as the chamber in which to produce the aerosol. While the nebulization of TNT from the above suspensions will produce an aerosol cloud of TNT particles in the 1- to 3- μ m range, several problems are evident. It appears that the concentrations needed to conduct the animal inhalation studies are so high that the TNT particles coalesce into larger particles and thus will not remain in airborne suspensions. Also, the TNT particles have a tendency to adhere to the surfaces of the glass jar. The net result of these problems is to greatly reduce the inhalable concentration of TNT within the chamber.

The second method of generation involved the dispersion of TNT using an air jet. As with the nebulization method, the problems of coalescence and adherence to the glass surfaces occurred. It would appear that the physicochemical properties of TNT may be at least a part of the problem because using the same method as described above, it is possible to produce an aerosol of talc which will remain in airborne suspension and will not adhere to surfaces in the manner observed with TNT.

2. Aerosolization by heating of TNT: TNT powder (20 to 28 μ m in size) contained in a glass petri dish was placed on an aluminum heating plate warmed by a 175-W heater. The temperature of the heater was controlled by a thermocouple connected to a temperature controller. A second thermocouple connected to a Pyrotest meter was placed in the petri dish to allow for direct temperature readings of the melted TNT liquid.

The experiments were carried out in a 0.5 m³ stainless steel exposure chamber. The heating device was placed in the bottom of the chamber, and the airflow entered the chamber at a point below the heating device. The airflow (50 liters/min) was controlled by a rotameter-type flowmeter. Samples for analysis of TNT concentration in the chamber were drawn through a Millipore filter (0.8- μ m pores) by a vacuum pump connected to a flowmeter to control the volume of the air sample withdrawn from the chamber. The TNT was eluted from the filters with toluene and analyzed by gas chromatography. Samples were collected with an impaction device and examined by light microscopy to determine particle size.

Initial experiments confirmed that a TNT aerosol could be produced by heating the TNT to approximately 200°C. However, the actual chamber concentrations measured were only 10 to 20% of the calculated concentration, which was based on chamber airflow and the amount of TNT consumed during the experiments. The particle size of the TNT obtained by this method was principally in the 3- to 8- μ m size range.

In an effort to determine the reason for the discrepancy between actual and calculated chamber concentrations, several experiments were conducted. To ensure that the chamber airflow was correct, the flowmeter was recalibrated using an Autotronics 100-SSX airflow transducer. This showed the flowmeter calibration to be correct; therefore, the airflow was not the

source of discrepancy in concentrations. Other possibilities considered included the possible generation of TNT vapor or TNT particles of smaller size that would not be captured on the Millipore filter sampling system. To test these possibilities, the chamber sampling was performed by passing chamber air samples through a vessel containing toluene to capture any TNT vapor or very small TNT particles. The concentrations obtained by this method were the same as those using the Millipore filter system. This suggests that TNT vapor or small TNT particles could not account for the discrepancy in concentrations.

As with the experiments using particles obtained with the jet pulverizer, there was a problem with TNT adhering to the walls of the exposure chamber. Also, after each experiment with the heating method, deposits of TNT dust were found in the bottom cone of the exposure chamber. In retrospect, it appears that the TNT produced by the heating method was of sufficiently high concentration to result in coalescence of particles to form larger particles which settled to the bottom of the chamber, resulting in reduced concentrations of TNT in the chamber environment. The presence of TNT particles primarily in the 3- to 8- μ m range might be a further indication of coalescence. Earlier small-scale studies of the heating method had produced particles in the 1- μ m size range.

C. Discussion

It appears that methods are available which can produce 1- to 3- μ m TNT particles and generate TNT aerosol. However, the problem in the present study appears to be the necessity for producing extremely high concentrations of TNT which are suitable for metabolic studies. We have estimated that TNT concentrations of 1 to 2 g/m³ would be needed to produce levels of TNT in the experimental animals that could be detected by available analytical methods. Production of TNT concentrations of this high magnitude was not possible using the methods described herein.

An alternative approach was the study of TNT disposition and metabolism after direct instillation into the trachea. This method has been used successfully by different investigators to study the toxicity and metabolism of various environmental chemicals, especially polycyclic aromatic hydrocarbons.⁶⁶⁻⁶⁸ While this method is not an inhalation exposure in the strictest sense, it does allow for the absorption of TNT via the lung. In this way, the metabolism of the TNT can be studied under conditions in which the TNT passes through the lung prior to entry into the bloodstream. Therefore, it would appear that a valid comparison or at least approximation of TNT metabolism by oral and pulmonary absorption could be made.

V. DISPOSITION STUDIES

A. Methods

1. Oral administration: Four rats, seven or eight mice, and three rabbits and dogs of both sexes were used in these studies. The animals were fasted overnight before receiving single oral doses of ^{14}C -TNT. Rats and mice received ^{14}C -TNT doses of 100 mg/kg dissolved in 10 ml/kg (rats) or 25 ml/kg (mice) of oil. Rabbits and dogs received ^{14}C -TNT doses of 5 mg/kg body weight dissolved in 1 ml/kg oil. The dosing solutions were prepared by dissolving the appropriate amounts of nonlabeled and ^{14}C -labeled TNT in peanut oil. A fresh dosing solution was prepared for each experiment to avoid possible decomposition. When not in use, the dose was stored refrigerated at 4°C. Aliquots of each solution were counted to determine the amounts of radioactivity. All dosing solutions contained $\cong 25 \mu\text{Ci}$ of the labeled compound per kilogram body weight.

After dosing, the animals were placed in individual stainless steel metabolism cages for the separate collection of urine and feces and were given food and water ad libitum. After 24 hr, the animals were anesthetized with ether (rats and mice) or sodium pentobarbital (40 mg/kg, i.p. for rabbits; and 30 mg/kg, i.v. for dogs). Blood was collected, and the following tissues and organs were removed, weighed, and analyzed for radioactivity:

Liver	Brain
Kidneys	Skeletal muscle
Lungs	Fat (retroperitoneal)
Spleen	GI tract plus contents

2. Dermal application: The fur on the back of the test animals was removed with an electric clipper. The day following clipping, the ^{14}C -TNT solution was spread over the depilated areas (2 to 4 cm² in mice, 8 to 10 cm² in rats, 150 to 200 cm² in rabbits, and 200 to 300 cm² in dogs). The doses applied were 50 mg/kg of TNT containing $\cong 25 \mu\text{Ci/kg}$ of ^{14}C -TNT in 2 ml/kg of peanut oil for rats; and in 5 ml/kg for mice. Rabbits and dogs were treated with either 5 or 50 mg/kg of TNT containing $\cong 25 \mu\text{Ci/kg}$ of ^{14}C -TNT in 0.5 ml/kg of peanut oil. Concurrent experiments were performed in animals treated orally with the same dose of ^{14}C -TNT and housed under the same conditions. Before being placed in metabolism cages, a plastic collar was placed around the neck of each mouse, rabbit, and dog to prevent them from grooming their fur. After dosing, rats were placed in individual restrainers, mice in small glass metabolic cages, and rabbits and dogs in steel metabolic cages. Urine and feces were collected separately for 24 hr. Blood samples were obtained from the tail veins of rats at 4, 8, and 24 hr after dosing. After 24 hr, the animals were anesthetized with ether (rats and mice) or with sodium pentobarbital (rabbits and dogs). Blood samples were collected, and the animals were killed for tissue sampling as described for the oral studies. Skin including the site of application was not retained for analysis.

The dermal and oral studies were performed with three (oral) and six (dermal) rats of both sexes, eight (oral) and seven (dermal) male mice, three (oral) and four (dermal) male rabbits, three (oral) and three (dermal) male dogs. In addition, limited studies were performed using four male rabbits (two oral and two dermal) and two male dogs (one oral and one dermal) which were treated with higher (50 mg/kg) doses of ^{14}C -TNT.

3. Intratracheal instillation: Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.), then tracheotomized with polyethylene tubing (PE-210). The femoral artery was cannulated with PE-50 tubing for collection of blood samples.⁶⁹ After the rats were allowed a 10- to 15-min recovery period, 50 mg/kg of TNT containing $\approx 25 \mu\text{Ci/kg}$ of ^{14}C -TNT was administered either orally or intratracheally. The TNT with particle size of 1 to 3 μm was suspended in a volume of 0.5 ml/kg of methylcellulose. Blood samples (0.2 to 0.3 ml) were collected for subsequent analysis. At the end of 4 hr, the rats were sacrificed, and tissues and bladder urine were collected for radioactivity analysis (see oral administration).

Since a major portion of the intratracheally administered TNT dose was recovered in the GI tract, some experiments were performed in rats which had the common bile duct cannulated with PE-10 tubing.⁶⁹ Bile was collected at different time periods after dosing and sampled for analysis. Blood samples were also collected, and the rats were sacrificed at 4 hr for tissue sampling.

4. Sample preparation and analyses: Feces and GI tract plus contents were weighed and homogenized separately in 10 volumes of ethanol:water (20:80) in a Waring blender. Duplicate aliquots of the homogenates (500 μl), whole blood (100 μl), and tissues (50 to 120 mg) were used for analysis. These samples were processed by heating in a shaking water bath at 70°C for 30 min with 0.2 ml of 70% perchloric acid and 0.4 ml of 30% hydrogen peroxide, then cooled and mixed with scintillation cocktail. (Preliminary studies indicated good recoveries when this technique was used for processing tissues and excreta.)^{70,71} Volumes of urine and cage rinse were measured, and aliquots (100 μl) were mixed with scintillation cocktail. Samples were analyzed in duplicate whenever possible. Phase Counting Solution (PCS, Amersham Corporation, Arlington Heights, Illinois) was used as the scintillation cocktail.

5. Radioactivity measurements: The samples were cooled for a minimum of 24 hr before counting in a liquid scintillation counter (Packard Tricarb Model 3375). Correction for background was carried out automatically on the counter. Background determinations were obtained by averaging the natural counts of several tissue homogenates from nontreated animals. The counting efficiency was determined using the automatic external standard (AES) method. An AES versus efficiency curve was prepared by processing a quench curve set through the counter under the conditions used throughout the experiment. Assays not within $\pm 10\%$ of the mean of the duplicates were reassayed in duplicate except when the sample was not available or when radioactivity counts were low and nonsignificant, i.e., less than two times the background.

6. Data processing and analysis: Carbon-14 contents in blood and tissues were presented in terms of microgram equivalents per milliliter (blood and bile) or gram (tissues) and percentage of the dose administered to each animal. Microgram equivalents per milliliter of blood and bile were also presented in graphic form. The means \pm standard errors were calculated for each test group with a programmable (Monroe) calculator. The significance of the data was determined by the two-tailed Student's t test. Significant differences were indicated when $p < 0.05$.

B. Results

1. Oral studies: The disposition of orally administered ^{14}C -TNT was studied in male and female rats, mice, rabbits, and dogs. No attempt was made to examine the radioactivity in the expired air since earlier studies performed at MRI* have demonstrated that only a negligible amount (0.1%) of the administered ring- ^{14}C -labeled TNT dose was eliminated by this route.

a. Rats: Recovery of radioactivity in tissues and excreta of rats is summarized in Table 1. At the end of 24 hr, a total of 52.7% of the administered dose was recovered in the urine, 8.1% in the feces, and 29.8% remained in the GI tract of male rats. Blood, liver, kidney, and spleen demonstrated high concentrations of radioactivity. The distribution and excretion of TNT and its metabolites in female rats were similar to that in male rats. During the same period the female rats excreted 64.6% of the dose in the urine and 2.1% in the feces while 33.9% remained in the GI tract. Urine of both males and females was bright red.

b. Mice: Table 2 summarizes the tissue distribution and excretion of radioactivity in mice treated orally with ^{14}C -TNT. In 24 hr, male mice excreted 41.9% of the administered dose in the urine and 22.0% in the feces; 13.5% remained in the GI tract. The females eliminated 42.9% in the urine and 9.0% in the feces; 7.4% was recovered in the GI tract. Tissues of female mice demonstrated lower radioactivity than those of males. This difference, which was statistically significant only in blood, liver, and kidney, is probably due to the low recovery in the females. Urine of mice had a bright red color similar to that of rats.

c. Rabbits: The rabbits excreted most of the administered radioactivity in the urine (66.3% of the dose in males and 78.9% in females).

* Lee, C. C., J. V. Dilley, J. R. Hodgson, D. O. Helton, W. J. Wiegand, D. N. Roberts, B. S. Andersen, L. M. Halfpap, L. D. Kurtz, and N. West. Mammalian toxicity of munition compounds: Phase 1. Acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism. United States Army Medical Research and Development Command, Midwest Research Institute Report No. 1, NTIS No. AD-B011, 150 (1975).

Feces contained 1.8% in both males and females. Recoveries in the GI tract averaged 7.5% in males and 4.7% in females (Table 3). Most tissues contained only small amounts of radioactivity. Liver, kidneys, and especially lungs had higher ^{14}C levels than did blood; lungs contained 9 times (males) or 14 times (females) the levels in blood. Rabbit urine did not contain the red pigment which was characteristic of the urine of rats and mice.

d. Dogs: Table 4 summarizes the tissue distribution and excretion of radioactivity in dogs after oral administration of ^{14}C -TNT. In males, 55.9% of the dose was excreted in the urine, 5.4% was recovered in the feces, and 10.0% in the GI tract. Females eliminated 60.2% of the dose in the urine and 16.8% in the feces while 4.4% remained in the GI tract. Expressed as percentages of the administered doses, dogs contained higher residual radioactivity than did rats, rabbits, or mice. Similar to rabbit urine, dog urine did not contain a red pigment.

A comparison of the tissue-to-blood concentration ratios in rats, mice, rabbits, and dogs at 24 hr after oral dosing with ^{14}C -TNT is shown in Table 5. High tissue-to-blood ratios were noted in liver (four species) and occasionally in kidneys and lungs (mice and rabbits). Rabbit lungs contained 9 times (males) or 14 times (females) higher ^{14}C levels than did blood. Low ratios (less than 1) were generally noted in brain and muscle and occasionally in lungs (rats).

2. Dermal studies: The absorption, tissue distribution, and elimination of ^{14}C -TNT was studied in male and female rats, male mice, male rabbits, and male dogs after dermal application. Concurrent experiments were performed in animals treated orally with the same dose of ^{14}C -TNT and housed under the same conditions used for the dermal application experiments. No attempt was made to measure the radioactivity on the site of dermal application.

a. Rats: Both male and female rats absorbed TNT after dermal application. Radioactivity in the blood increased with time following dermal application and continued to increase until at least 24 hr after dosing. After oral administration, on the other hand, the highest radioactivity in the blood was seen at 8 hr (Table 6). After both treatments, the urine of rats was red.

At the end of 24 hr, the distribution of radioactivity in blood, lung, spleen, brain, and muscle was comparable after both oral and dermal administration of ^{14}C -TNT to male rats (Table 7). However, the fat contained a higher concentration of radioactive TNT after dermal application, and the liver and kidney contained higher concentrations of radioactivity after oral dosing. Most of the absorbed radioactivity was excreted in the urine, averaging 17.4% of the administered dose after dermal application and 59.5% after oral administration. Radioactivity was also recovered in the feces and GI tract, averaging 1.3 and 3.1%, respectively, after dermal application; and 10.7 and 20.2%, respectively, after oral treatment.

In female rats, the distribution of radioactivity was similar to that in male rats (Table 8). At the end of 24 hr, the distribution of

radioactivity in blood and most tissues was comparable after oral and dermal administration. Fat contained greater levels of radioactivity after dermal application, and liver contained more radioactivity after oral dosing. These differences, however, were not significant. At the end of 24 hr, a total of 14.6, 2.5, and 6.4% of the dermally applied radioactivity was recovered in the urine, feces, and GI tract, respectively. After oral administration, recoveries from urine and GI tract were significantly greater, averaging 42.5 and 35.3%, respectively, of the administered dose; in the feces, recovery was 2.1% after oral administration.

b. Mice: After dermal application of ^{14}C -TNT to male mice, absorption occurred readily. At the end of 24 hr, 22.7, 14.2, and 3.6% of the administered dose was recovered in the urine, feces, and the GI tract, respectively (Table 9). After oral dosing, the recovered radioactivity averaged 59.1, 24.1, and 10.2%, respectively; these recoveries were significantly larger than those after dermal application. Radioactivity remaining in most tissues was comparable after both routes of administration. As in rats, ^{14}C content in fat was higher after dermal application, whereas the radioactivity in liver was higher after oral dosing. After both routes of administration, the urine had the same red color that was observed in urine of TNT-treated rats.

c. Rabbits: A dose of 5 mg/kg of ^{14}C -TNT was administered to groups of male rabbits dermally or orally. This dose was the same as was used in the oral studies performed earlier. However, the volume of vehicle (peanut oil) was reduced to 0.5 ml/kg. After dermal application, the major portion of the absorbed radioactivity was eliminated in the urine, averaging 52.9% of the dose (Table 10). In addition, 7.8% of the dose was recovered in the feces and 5.8% in the GI tract. After oral dosing, recoveries in the urine, feces, and GI tract averaged 68.1, 5.4, and 19.7%, respectively. Radioactivity in blood and residual bile was higher after oral administration, whereas radioactivity in kidney, lung, brain, and fat was higher after dermal application.

An additional study was conducted in groups of male rabbits treated dermally or orally with a 50 mg/kg dose of ^{14}C -TNT. This study was performed in order to (a) acquire larger amounts of TNT metabolites in the urine for TLC analysis; (b) compare the profiles of metabolites in different species after administration of the same dose of ^{14}C -TNT; and (c) examine the effect of increasing dose on the disposition and metabolism of TNT. Apparently the high dose, 50 mg/kg, did not alter the absorption, distribution, and excretion of TNT when compared with the low dose, 5 mg/kg (Table 11). However, the number of rabbits used (two per treatment) was too small to make a statistical comparison between the different dose levels or treatments. The red pigment excreted in the urine of rats and mice treated with a 50 mg/kg dose of TNT was not found in the urine of rabbits treated with the same dose, although it was reported earlier⁵⁶ that a red pigment was excreted in the urine of rabbits treated with higher and repetitive doses of TNT.

d. Dogs: A dose of 5 mg/kg of ^{14}C -TNT was administered to male dogs orally or applied dermally. The absorption of TNT after dermal

application was significantly lower than in rabbits and mice and slightly lower than in rats. At the end of 24 hr, 11.7% of the dose was recovered in the urine, 1.7% in the feces, and 1.7% in the GI tract (Table 12). After oral administration, 70.5% of the dose was excreted in the urine and 9.0% in the feces while 14.6% remained in the GI tract. Radioactivity in blood, liver, kidney, spleen, muscle, and residual bile was higher after oral administration, whereas radioactivity in fat was higher after dermal application.

To obtain preliminary information on the effect of dose on TNT absorption and elimination in dogs, ^{14}C -TNT was administered orally and dermally at a dose of 50 mg/kg. One animal was dosed by each route. Absorption and excretion of TNT appeared similar in both dose levels studied (5 and 50 mg/kg), although blood content (percent of dose) was higher after the high dose of TNT (Table 13).

After administration of ^{14}C -TNT to dogs by both routes, radioactivity was concentrated in the residual bile and liver (Table 14). Radioactivity levels were also high in the residual bile and liver of rabbits; levels in bile were considerably higher than in the blood. The concentration ratios (bile/liver and bile/blood) of radioactivity were higher for dogs than for rabbits (Table 14).

The tissue-to-blood concentration ratios at 24 hr after oral or dermal dosing of ^{14}C -TNT are shown in Table 15. Liver, kidney, lung, and occasionally spleen showed ratios higher than 1.0, while brain and muscles demonstrated ratios lower than 1.0. The ratios in fat differed after both routes of administration and were lower than 1.0 after oral administration and higher than 1.0 after dermal treatment.

3. Intratracheal studies: As an alternative approach to the exposure of rats to TNT by inhalation, studies were performed in which ^{14}C -TNT was instilled directly into the trachea of rats. A suspension of ^{14}C -TNT was delivered through a cannula placed surgically into the trachea in order to ensure that the precise dose was administered. Attempts to let the anesthetized rats recover failed. Therefore, subsequent experiments were performed under pentobarbital anesthesia. Although it was appropriate to administer the 100 mg/kg dose of ^{14}C -TNT used in the oral experiments described earlier, limitations on the quantities of powder and vehicle instilled into the trachea necessitated reducing the dose to 50 mg/kg and the volume of the vehicle to 0.5 ml/kg. Initially, 0.2% solution of Tween 80 was used to suspend ^{14}C -TNT, but it was found that the use of a solution of 0.5% methylcellulose was satisfactory. Concurrent experiments were performed in rats treated orally with the same dose of ^{14}C -TNT under the same experimental conditions.

Preliminary experiments performed in rats dosed intratracheally indicated a fast rate of absorption of TNT from the trachea and disappearance of TNT from blood. Therefore, the experiments were terminated 4 hr after dosing. Since the GI tracts of the intratracheally treated rats contained considerable amounts of radioactivity, some experiments were performed in which the bile ducts were cannulated for collection of bile. The survival

rate during the intratracheal instillation of TNT was fair; more than 80% of the treated rats survived the experiment. Some of the surviving rats had slight difficulty in breathing for about 10 min after dosing.

After oral administration of ^{14}C -TNT, radioactivity appeared in the blood of male rats within 15 min (Figure 2). The radioactivity in blood continued to increase for 60 min and maintained a constant level thereafter during the 4-hr experiment. After intratracheal instillation, absorption was faster, greater, and more uniform with less individual variation than was noted after oral administration. Orally treated male rats excreted 10.7% of the administered dose in the urine and 11.6% in the bile from bile duct-cannulated rats (Table 16). The amounts excreted in the urine and bile were higher after intratracheal instillation, averaging 17.5 and 19.8% of the dose, respectively. As shown in Figure 3, biliary excretion reached a peak 30 min after oral administration and remained constant thereafter. After intratracheal instillation, biliary excretion quickly reached a peak at 30 min and decreased gradually thereafter. Cumulative excretion of radioactivity in bile is shown in Figure 4. Urinary and biliary excretions were generally lower in female rats. Excretion of radioactivity in urine and bile averaged 8.4 and 9.7% of the administered dose, respectively, after oral administration; and 12.7 and 14.5%, respectively, after intratracheal instillation (Table 17). In rats without biliary cannula, excretion in the urine was higher (Tables 16 and 17). In all cases, urine was red and bile was dark orange.

High concentrations of radioactivity were found in tissues, especially in the blood, liver, kidney, lung, fat, and GI tract (Tables 16 and 17). In general, radioactivity in most tissues was higher after intratracheal instillation than after oral administration. Levels of ^{14}C in blood and tissues of female rats were about two times higher than in the males. Radioactivity was concentrated in the bile; bile-to-liver and bile-to-blood concentration ratios were high after both routes of administration (Table 18).

A comparison of the tissue-to-blood concentration ratios at 4 hr after oral or intratracheal administration of ^{14}C -TNT is shown in Table 19. Fat-to-blood ratios were high (3.2-5.3) in males and females after both routes of administration. The lungs of male rats also showed high ratios after oral and intratracheal dosing. The ratios in liver were about 1.0 in males and less than 1.0 in females. Ratios less than 1.0 were demonstrated in spleen, brain, and muscle of all treatment groups.

C. Discussion

There are three possible routes for TNT to enter the body: ingestion, absorption through the skin or via the lung, or any combination of these, depending on the type of exposure involved. Earlier studies have suggested that the skin is the chief avenue of absorption.⁴⁶ Voegtlin et al.⁴⁷ have demonstrated that in humans, skin absorption takes place readily through the hands, neck, and face; oily skin and sweat favor absorption.

Although some experiments have demonstrated that TNT is absorbed when introduced as dust in the lower air passages, Puntam and Herman⁴⁶ suggested that intoxication via the respiratory tract rarely, if ever, occurs.

1. Oral absorption: During exposure to TNT, the powder may be ingested by mouth and gain access to the stomach. TNT workers have complained about a bitter taste in their mouth. When two human subjects received daily doses of 1 mg/kg of TNT for four successive days, 3% of the total amount of TNT administered was recovered from the urine in the form of 2,6-dinitro-4-aminotoluene.⁵⁰ Experimentally, guinea pigs fed oral doses of TNT with milk developed diarrhea, and poisoning symptoms were apparent for 3 to 14 days.⁵¹

The present study demonstrates that TNT is readily absorbed after oral administration to rats, mice, rabbits, and dogs. It appears that the rabbits and dogs absorb more TNT than do rats and mice. However, the extent of absorption can be only approximated from our recovery data since the extent of biliary excretion and enterohepatic circulation was not studied. Radioactivity recovered in the GI tract represents a balance between absorption, biliary excretion, and intestinal reabsorption.

2. Dermal absorption: TNT was reported to be absorbed through the intact skin of swine as indicated by the presence of 2,6-dinitro-4-aminotoluene in urine.⁵² Also, Haythorn reported that guinea pigs and rabbits rubbed repeatedly with 10% TNT in lanolin showed a positive Webster's test (a test introduced in 1916 by Webster which has been used to detect TNT in human urine in cases of intoxication) and liver lesions.⁵³ However, when Haythorn rubbed TNT powder on his arm for several consecutive days, he could not demonstrate a positive Webster's test and did not feel any ill effects.⁵³ In another study,⁵⁴ powdered TNT was rubbed into the palms of two human subjects and kept under rubber gloves for 8 hr; traces of the metabolite 2,6-dinitro-4-aminotoluene were found in the urine collected during the exposure and for 15 hr thereafter.

The dermal experiments performed in the present study confirm the potential absorption of TNT through the skin. TNT was most readily absorbed by rabbits, followed by mice, rats, and finally dogs. The majority of the administered dose was recovered in urine, feces, and GI tracts. In all species, the total elimination of the administered radioactivity was lower following dermal application than after oral treatment.

3. Pulmonary absorption: Absorption through the respiratory system has been previously examined by Haythorn.⁵³ When guinea pigs were exposed to fumes of volatilized TNT for 3 hr/day for 30 days, no lesions ascribed to TNT were observed, but the animals died from the heat used to volatilize TNT. In another series of experiments, TNT powder was introduced into the lungs of experimental animals, but no toxicity developed. This led Haythorn to conclude that the lung is unimportant as a route of intoxication from TNT. Later, however, Von Oettingen and his colleagues administered TNT to dogs by insufflation and demonstrated that 75% of the dose was absorbed from the respiratory tract.⁴⁸

In the present study, extensive absorption was demonstrated when TNT suspension was instilled in the rat trachea. The pharmacokinetic behavior of TNT after intratracheal instillation was comparable to the behavior usually observed after intravenous administration of other xenobiotics. The rate of absorption was considerably faster than after oral administration, and blood levels also decayed at a faster rate. Intratracheal instillation of TNT was not studied in mice, rabbits, or dogs. If the results of the rat study can be extrapolated to other experimental animals and humans, it suggests that, when TNT powder reaches the respiratory tract, absorption will occur at a fast rate.

The dermal and oral studies were terminated after 24 hr, but the intratracheal instillation experiments were terminated 4 hr after dosing. Therefore, no data are available for direct comparison between the intratracheal and dermal routes. Blood sampled at 4 hr after dermal treatment of male rats showed considerably lower levels of radioactivity than the levels obtained after intratracheal dosing. However, blood levels continued to increase between 4 and 24 hr after dermal application; after intratracheal administration these levels would probably decrease. Therefore, the available data indicate that the rates of absorption and elimination of TNT are highest after intratracheal instillation and lowest after dermal application.

4. Tissue retention: Voegtlin et al. believed that TNT is retained in the body for a considerable period of time, as indicated by the progressive anemia after single doses of TNT and by the slow recovery of the animals.⁴⁷ However, when Von Oettingen and co-workers administered TNT to dogs by insufflation 5 days/week for a period of 17 weeks, significant amounts of TNT or its metabolite, 2,6-dinitro-4-aminotoluene, were not found in any organ or tissue examined at the end of the study.⁴⁸ These authors concluded that TNT is not retained to any considerable extent in these organs. Conclusions from these early studies regarding storage or excretion of TNT and its metabolites are hampered by the insensitivity of the methods used to examine the presence of either TNT or the reduced metabolites, aminotoluenes.

Although the experiments in the present studies were not extended beyond 24 hr, there is indication that retention in tissues of the four species examined is not extensive. The extent of retention and storage of radioactivity did differ, however, between species and between routes of administration. In addition, the patterns of radioactivity in tissues of rats were different when examined at 4 hr compared to 24 hr after treatment. The present studies were performed after administering single doses of TNT. It may be possible that after repetitive dosing the amounts of TNT and/or its metabolites retained in the various tissues would differ from amounts retained after single doses.

5. Urinary excretion: Based on Webster's test, it was suggested that the urine was the main route of excretion for TNT. Studies by Lamberg and Callaghan indicated that 20% of a single oral dose of TNT was excreted in the urine of rats as diazotizable aromatic amino compounds.⁵⁵ Human volunteers excreted ~ 40% of small oral doses of TNT as aromatic amino compounds in the urine. In other experiments, humans receiving 1 mg/kg/day of

TNT excreted about 3% of the ingested dose as 4-amino and 2,4-diamino products; concentration of these metabolites fell almost to zero within 24 hr after the last dose.⁵⁰ The present studies demonstrated that after oral administration of TNT to rats, mice, rabbits, and dogs, large portions of the administered doses were excreted in the urine. After intratracheal instillation, extensive elimination occurred through the urine and the GI tract. After dermal application, dogs and rats excreted only small portions of the doses in the urine and feces within 24 hr. On the other hand, rabbits and mice excreted large portions of the doses in the urine and feces; these differences in excretion may be due solely to differences in the rates of absorption.

Urine from humans and some experimental animals given TNT contained a red pigment which was believed to be a metabolic product of TNT.⁵⁶ Rats treated with α -TNT (2,4,6-TNT) or with 2,4,6-trinitrobenzyl alcohol excreted the red pigment; β -TNT (2,3,4-TNT), γ -TNT (2,4,5-TNT), and several other related TNT intermediates did not cause any changes in color of the urine.⁵⁶ In the present studies, the bright red color of urine was observed in rats and mice treated by the different routes of administration. Urine of dogs and rabbits treated orally or dermally contained no red pigment even after treatment with high doses (50 or 100 mg/kg). This seems to be in variance with the early observations by Channon et al., who reported the presence of red pigment in the urine of rabbits treated orally with TNT.⁵⁶ Their experiments, however, were repetitive and used higher doses of TNT.

6. Biliary excretion: Voegtlin et al. suggested that TNT was excreted in bile.⁴⁷ Haythorn, however, could not obtain a positive Webster's test in the feces of any animals given TNT by any route except orally.⁵³ The present experiments demonstrated that in all species examined biliary excretion plays a major role in the disposition of TNT as indicated by the radioactivity recovered in the bile of bile duct-cannulated rats or in the residual bile of dogs and rabbits. It was also indicated by the excretion of radioactivity in feces and GI tract after dermal application of TNT to rats, mice, rabbits, and dogs, or after intratracheal instillation in rats.

The enterohepatic recycling of TNT and/or its metabolites (excretion in bile followed by intestinal absorption and reexcretion in urine or feces) was suggested by the higher recovery of radioactivity in the urine of noncannulated rats than in urine of bile duct-cannulated rats. In addition, radioactivity excreted in the bile of rats was equal to or more than that excreted in the urine, whereas radioactivity excreted in the urine of noncannulated rats was more than that recovered in the feces and GI tracts. Radioactivity recovered in the GI tract after oral dosing represents a balance between absorption, excretion in bile, and intestinal reabsorption. After intratracheal administration, the recovered radioactivity represents the difference between biliary excretion and intestinal reabsorption; only a small portion of the dose appears to be excreted through the intestinal wall.

VI. METABOLIC STUDIES

A. Methods

1. Extraction and cleanup procedures: For the chromatographic separation of trinitrotoluene (TNT) and its metabolites, urine samples were extracted with either ethyl acetate or ether. The efficiency of the extraction was examined at the pH of the raw urine (pH 7-8 for rat, mouse, and dog urine, and pH 9-9.5 for rabbit urine); at pH 6, 7, or 8 by adding equal volumes of the corresponding phosphate buffer (0.2 M); and at pH 4 or 5 by treatment with acetate buffers (0.2 M). Urine samples were also extracted after adding varied amounts of dilute acid (hydrochloric or acetic) or alkalis (sodium hydroxide or carbonate). In addition, some urine samples were extracted successively with ethyl acetate under acidic, neutral, and basic conditions and the extracts were pooled. Early in the study, urine samples were treated with a strong acid (5 N hydrochloric acid) and heated for 1 hr at 100°C prior to the extraction procedure. This method was abandoned later in view of the demonstrated instability of many of the metabolic products of TNT under these conditions.

It was established that acidification with 0.1 N hydrochloric acid (1/10 of urine volume) before extraction with ethyl acetate resulted in the highest recovery. Therefore, the method routinely used involved the extraction of urine or bile samples with 10 volumes of ethyl acetate after acidification with 1/10 volume of dilute hydrochloric acid (0.1 N). The samples were mixed thoroughly for 3 min with a vortex mixer and centrifuged. The organic extracts were separated, dried with anhydrous calcium chloride, and filtered. To assess the recovery of extractions, aliquots of these extracts (0.5 ml) were placed in counting vials and counted in 10 ml of scintillation cocktail. This extraction procedure was repeated three times, and the extracts were pooled and evaporated in a rotary evaporator. The residues were dissolved in small volumes (0.1 to 0.2 ml) of methanol, ethyl acetate, or a mixture of both solvents (1:1), filtered through Millipore filters, and used for analyses with thin-layer chromatography and high performance liquid chromatography.

Some urine samples were lyophilized (freeze-dried), and the residues were dissolved in methanol, ethyl acetate, or a mixture of both solvents (1:1). These were centrifuged, filtered through Millipore filters, and used for chromatographic analysis. Additional urine samples were purified by using XAD-2 (Amberlite) resin. The urine was passed through the resin column, and the radioactivity was eluted with water followed by methanol. Radioactivity was counted in each fraction collected.

2. Enzyme hydrolysis: During the early studies, urine samples were acidified with 5 N hydrochloric acid and heated for 1 hr to hydrolyze the conjugated metabolites. This method was abandoned later after it was recognized that the nature of TNT metabolites might be altered by this treatment. The hydrolysis of the conjugated metabolites was carried out by incubation with excess β -glucuronidase (type II, Sigma Chemical Company,

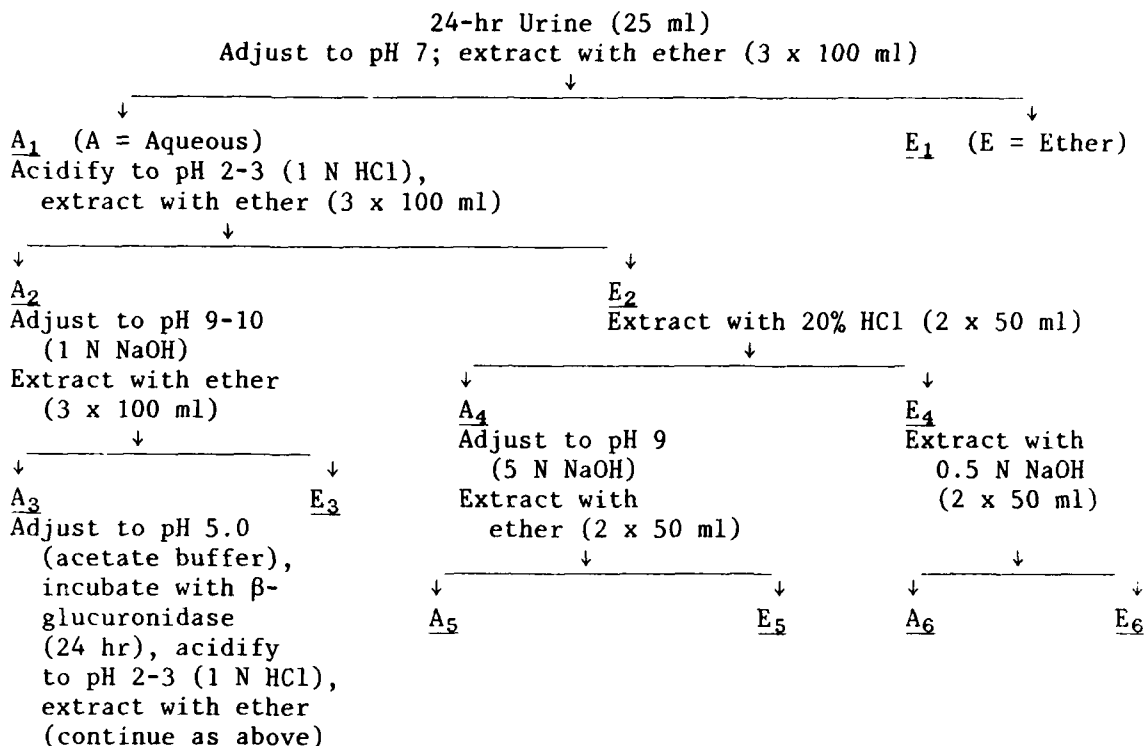
St. Louis, Missouri) at a final concentration of about 20,000 units/ml, after adjusting the urine samples to pH 5 with sodium acetate-acetic acid buffer (0.2 M). Some urine samples were also treated with aryl sulfatase (type V, Sigma) under the same pH conditions. For routine analysis, urine or bile samples (1 to 2 ml) were mixed with equal volumes of the buffer, and the mixtures were treated with 0.25 to 0.5 ml of liquid β -glucuronidase (type H-2, 100,000 units/ml) which also contains some aryl sulfatase. The reaction mixtures were incubated (37°C) under anaerobic conditions (N_2) for 24 hr in a Dubonoff shaking incubator (100 cycles/min). Urine incubated only with the acetate buffer served as control (to assess the nonenzymatic hydrolysis).

After incubation, the reactions were terminated by the addition of 0.5 ml of 0.1 N HCl and 10 ml of ethyl acetate which was used to initiate the extraction process. The aqueous layers were extracted further with two 10-ml portions of ethyl acetate. The extracts were pooled, dried with anhydrous calcium chloride, and filtered. The filtrates were evaporated under vacuum, and the residues were dissolved in 0.1 to 0.2 ml of methanol, ethyl acetate, or a mixture of both solvents (1:1). Portions of these solutions were used for the characterization of TNT metabolites with TLC or HPLC. Some urine samples were incubated with β -glucuronidase and analyzed with HPLC without prior extraction with ethyl acetate.

3. Fractionation of urinary metabolites: To simplify the chromatographic profile of TNT metabolites, separation of these metabolites into several major subgroups was attempted. Urine samples were subjected to a series of extractions with ether under different pH conditions. Several modifications were made during the development of the procedure, shown in the following diagrammatic scheme. The ether extracts were dried with anhydrous sodium sulfate, filtered, and evaporated under vacuum. The residues were prepared for TLC analysis. The remaining aqueous layer (A_3) was acidified and incubated with β -glucuronidase, and the extraction process was repeated. To calculate recoveries, portions of the ether and aqueous solutions were placed in scintillation vials, mixed with 10 ml of scintillation cocktail, and counted.

For comparison, a mixture of TNT and the nine available standards of potential metabolites listed below was subjected to the same extraction procedure. The lack of solubility of some of these metabolites (especially the azoxytoluene derivative) in water necessitated the addition of small amounts of methanol (5% of the total volume). After extraction, the different ether fractions were evaporated, and the residues were subjected to TLC analysis.

1. Trinitrotoluene (TNT)
2. Trinitrobenzyl alcohol
3. Trinitrobenzoic acid
4. 4-Amino-2,6-dinitrotoluene
5. 2-Amino-4,6-dinitrotoluene
6. 4,6-Diamino-2-nitrotoluene
7. 2,6-Diamino-4-nitrotoluene
8. 4-Hydroxylamino-2,6-dinitrotoluene
9. 2-Hydroxylamino-4,6-dinitrotoluene
10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene



4. Thin-layer chromatography: Precoated silica gel G plates (0.25 mm thickness on aluminum support) purchased from Brinkmann Instruments, Inc. (Des Plaines, Illinois) were used routinely throughout the study. Samples of urine or bile extracts and raw or lyophilized urine were spotted (\cong 2.0 cm from the bottom of the plate) and developed for 15 to 16 cm. The following solvent systems were tested for their ability to separate TNT and some reference standards of potential metabolites:

Solvent I:	n-Butanol:acetic acid:water (10:1:1, v/v/v)
Solvent II:	Benzene:acetic acid (9:1, v/v)
Solvent III:	Toluene:benzene:hexane (10:10:5, v/v/v)
Solvent IV:	Ethyl acetate:n-heptane (9:1, v/v)
Solvent V:	Benzene:ethyl acetate (4:1, v/v)
Solvent VI:	n-Butanol:acetic acid (10:1, v/v)
Solvent VII:	Benzene:acetic acid (4:1, v/v)
Solvent VIII:	Benzene:ethyl acetate (2:1, v/v)
Solvent IX:	Toluene:acetic acid (4:1, v/v)

For routine analysis, solvent systems I and VII were used for developing different TLC plates. Due to the Occupational Safety and Health Administration (OSHA) restrictions on the use of benzene, solvent system VII was replaced later with system IX, which contains toluene. Samples of pure TNT and nine reference standards of potential metabolites (the alcohol,

acid, monoamines, diamines, hydroxylamines, and azoxytoluene) were spotted and developed alongside the extracted material. After development, the plates were air-dried, cut into 1.0 cm zones (unless otherwise specified), and placed in scintillation vials. Ten milliliters of scintillation cocktail (PCS, Amersham) were added, and the vials were mixed thoroughly with a vortex mixer and counted.

Occasionally, the dried plates were sprayed with Bratton-Marshall reagent to detect the presence of arylamines.⁷² Nitro compounds were detected by using 5% diphenylamine in absolute ethanol.⁷³ The presence of hydroxylamines was examined by spraying the plates with triphenyltetrazolium chloride (TTC) in the presence of alkali or with Benedict's reagent.⁷⁴ Hydroxylamines developed a purple red color in TTC and were mildly reducing to Benedict's reagent.

5. Gas-liquid chromatography (GLC): A Hewlett-Packard Model 5736-A gas chromatograph equipped with a flame ionization detector was used for GLC. Two columns, differing in polarity, were tested for the separation of TNT and potential metabolites. Column A was a stainless steel column (0.125 in. ID x 3 ft) packed with 10% UCW-872 on WAW-DMCS (80-100 mesh); column B was a glass column (0.25 in. ID x 4 ft) packed with 1.5% DC LSX-30295 and 1.5% GE-XE-60 on Gas Chrom Q (60-80 mesh).

6. High performance liquid chromatography: A Waters Associates liquid chromatograph equipped with a Model U6K injector, Model 660 programmer, Model 6000 pumps, and Model 440 detector (254 nm, ultraviolet) was used. The following four HPLC systems were examined for their ability to separate TNT and some of its potential metabolites. System 1 was regular phase chromatography, but systems 2 to 4 utilized the counter-ion reverse phase chromatography. System 4 was selected for the analysis of urinary metabolites.

System 1: Column: μ Porasil, 300 x 4 mm ID
Solvent: Isocratic tetrahydrofuran (THF):hexane (5:95)
for 5 min, THF:hexane (5:95 to 45:55) in
15 min, after 30 min programmed from THF:
hexane (45:55 to 70:30) in 5 min
Flow rate: 1 ml/min

System 2: Column: C₁₈ μ Bondapak, 300 x 4 mm ID
Solvent: A--0.005 M tetrabutylammonium hydroxide
(TBA) in water adjusted to pH 7.5
with phosphoric acid
B--0.005 M TBA in tetrahydrofuran, to which
is added the same amount of phosphoric
acid as used in solvent A
Flow rate: 1 ml/min
Program: 0 to 100% B in 15 min
Program type: 6 (linear)

System 3: A modification of system 2 to obtain better resolution.

Column: C₁₈ μ Bondapak, 300 x 4 mm ID

Solvents: A--0.005 M TBA in water adjusted to pH 7.5 with phosphoric acid

B--0.005 TBA in methanol to which is added the same amount of phosphoric acid used in A

Flow rate: 1 ml/min

Program: Isocratic 25% B for 30 min, then 25 to 100% B in 25 min

Program type: 9 (nonlinear)

System 4: A modification of system 3 developed to retain the same resolution but to allow the polar urine components (excipients) to be eluted at the solvent front before elution of the compounds of interest. The following modifications were made in system 3:

Program: Isocratic 15% B for 20 min, then 15 to 100% B in 30 min

Program type: 6 (linear)

Samples of urine or urine extracts (20 to 100 μ l) were injected into the system, and fractions of 0.4 to 0.8 ml were collected for liquid scintillation counting.

B. Results

1. Extraction and cleanup procedures: Extraction of the urine with ether or ethyl acetate without adjusting the pH resulted in very low recovery of radioactivity. The recovery was not greatly improved by treating the mixture with 0.2 M phosphate buffers (pH 6, 7, or 8), but addition of acetate buffer (pH 4.0 or 5.0) increased the amounts extracted. A considerable increase in the recovered radioactivity was achieved by the use of dilute (0.1 N) HCl (1/10 volume of urine sample) followed by extraction with ethyl acetate or ether. No additional radioactivity was obtained when a stronger acid was used; in fact, this reduced the amount of extractable radioactivity in the organic solvent. In alkaline medium (NaOH or Na₂CO₃), very little radioactivity was recovered. When urine samples were extracted successively with ethyl acetate under neutral, alkaline, and acidic conditions and the extracts combined, the recovery was only slightly higher than that obtained under mild acid conditions. The method routinely used for urine or bile extraction involved the acidification with 0.1 N HCl (1/10 volume) and extraction with ethyl acetate three times. A large portion of the radioactivity was, however, still unextractable under these conditions.

When urine samples were lyophilized and the residues dissolved in methanol, ethyl acetate, or a mixture of both solvents, it was noted that a

major portion of the radioactivity was not soluble. Attempts were also made to purify urine samples by passing them through a column of XAD-2 (Amberlite) resin and eluting with water followed by methanol. It was hoped that the radioactivity would be eluted only with methanol. This, however, was not the case; the major portion of radioactivity was eluted with water. Therefore, this procedure was abandoned.

2. Enzyme hydrolysis: Urine samples from rats, mice, rabbits, and dogs were hydrolyzed by incubation with β -glucuronidase (free of aryl sulfatase) in the presence of acetate buffer, pH 5.0. At the end of incubation, samples were acidified with 0.1 N HCl and extracted with ethyl acetate. Considerable increases in the extractable radioactivity occurred after hydrolysis. These increases were not detected when incubations were performed in the presence of both β -glucuronidase and saccharo-1,4-lactone (10 μ m), an inhibitor of the β -glucuronidase enzyme. This indicated that the hydrolysis of the glucuronide conjugates was enzymatic. Incubation with aryl sulfatase did not increase the amount of extractable radioactivity. Routine hydrolysis was carried out with β -glucuronidase which contained some aryl sulfatase.

The amounts of ethyl acetate-extractable radioactivity from urine incubated with or without β -glucuronidase are shown in Table 20. The amounts of extractable radioactivity from urine of each species were not different after oral or dermal administration or after oral or intratracheal administration of TNT. Extractable radioactivity was also not different in male or female rats. Without incubation with β -glucuronidase, the urine from mice contained more extractable radioactivity than urine from rabbits, dogs, or rats. Incubation with β -glucuronidase increased the extractable radioactivity of urine samples from all species regardless of route of administration. The ratios of extractable radioactivity after incubation with β -glucuronidase to that without incubation with β -glucuronidase were low for urine from mice and high for urine from rats, rabbits, and dogs. These results suggest that the urine from mice contained small amounts of glucuronide conjugates of TNT metabolites and that the urine from rats, rabbits, and dogs contained large amounts of the conjugates.

Bile samples from rats, rabbits, and dogs were also extracted with ethyl acetate after incubation without or with β -glucuronidase. The results indicate that the amount of radioactivity extractable in ethyl acetate without hydrolysis was small in bile from the three species. Considerable increases in the extractable radioactivity occurred after incubation with β -glucuronidase, suggesting that bile contained large amounts of glucuronide conjugates. No major differences were demonstrated in the amounts extracted from bile of orally or intratracheally treated rats, and orally or dermally treated rabbits and dogs (Table 21).

3. Fractionation of urinary metabolites: Because of the closely related chemical structures, the similar solubilities, and the amphoteric nature of several metabolic products of TNT, complete separation of these metabolites would not be expected to occur by simple extraction. Therefore, a method was worked out to separate the metabolites in urine into several subgroups according to their solubility in organic and aqueous solvents at different pH conditions.

A mixture of TNT and nine potential metabolites was subjected to the extraction procedure. The organic fractions were analyzed with TLC to determine the recovery of each compound in each fraction. As shown in Figure 5, TNT and some potential metabolites were extractable to different extents in ether (E_1) before acidification. None of the trinitrobenzoic acid was extractable until after acidification. In this fraction (E_2), all the acid and most of the monoamines, hydroxylamines, alcohol, and azoxytoluene were recovered. Some of the diamines and TNT were also present. Most of the diamines, however, and some of the monoamines were present in the basic fraction, E_3 . When the E_2 fraction was extracted with 20% hydrochloric acid, all the monoamines and diamines present in this fraction were removed into the acid (A_4). Ether extraction of A_4 , after alkalization with NaOH, removed all the monoamines and diamines into E_5 . Compounds remaining in E_4 , which are the acid, most of the hydroxylamines, alcohol, and azoxytoluene, in addition to some TNT, were extracted with NaOH. The acid and most of the hydroxylamines were removed into the aqueous fraction, A_6 . The remaining ether extract (E_6) contained the alcohol, the azoxytoluene, some of the hydroxylamines and TNT.

Results from Figure 5b show that the E_1 fraction of the urine samples contained very low radioactivity, ranging from 4.6% in rat urine to 11.0% in dog urine obtained after oral administration of TNT. The extractable radioactivity was higher in urine of rats (10.7%) and dogs (16.1%) obtained after dermal application. The extractable radioactivity from urine of mice and rabbits remained about the same after both routes of administration. In all species, most of the radioactivity remained in the aqueous solution, A_1 . Acidification of this solution with dilute HCl increased considerably the recovery of radioactivity in the ether extract. Recoveries from urine of orally treated animals in the E_2 fraction ranged from 32.6% for rabbits to 51.4% for mice. The amounts extracted from urine of dermally treated animals ranged from 22.8% for dogs to 38.5% for mice and rabbits. About 40 to 60% of the initial radioactivity in urine samples remained in the aqueous solution, A_2 .

When this solution (A_2) was adjusted to pH 9-10 and extracted with ether, only small portions of the radioactivity were extractable (E_3). These ranged from 1.4 to 2.6% for urine obtained after oral administration and 0.6 to 1.9% for urine obtained after dermal application. The aqueous fractions, A_3 , which seemed to contain conjugated metabolites, were treated with acetate buffer (pH 5.0) and incubated with β -glucuronidase, then subjected to ether extractions as described above. The extraction performed after hydrolysis with β -glucuronidase was attempted with urine samples from rats and rabbits. However, after it was apparent that the metabolic profiles after β -glucuronidase hydrolysis were almost identical to those obtained without β -glucuronidase treatment, successive extraction and analysis of metabolites after hydrolysis were discontinued.

The radioactivity in the E_2 fractions was extracted with 20% HCl. Considerable portions of the radioactivity were transferred to the acid fractions (A_4). The amounts ranged from 7.3% in urine of rabbits to 14.3% in urine of mice obtained after oral administration, and from 5.1% in urine of

dogs to 11.8% in urine of mice obtained after dermal application. Most of the radioactivity, however, remained in ether (E_4). These averaged 24.6 to 37.8% of the activity in urine after oral administration and 17.7 to 27.5% in urine after dermal application. The aqueous solutions (A_4) were made alkaline (pH 9) and again extracted with ether. Only small portions (1.0 to 6.2%) of the metabolites were extractable in ether (E_5). Most of the radioactivity (3.9 to 10.3%) remained in the aqueous solutions (A_5). The radioactivity in E_4 fractions was subjected to additional extraction with 0.5 N NaOH. Large portions of the metabolites were removed into the alkaline solution (A_6) ranging from 16.7 to 31.8% (oral) to 13.3 to 22.6% (dermal). Smaller amounts remained in the ether fractions (E_6) ranging from 6.7 to 15.1% and 2.2 to 11.3% in urine obtained after oral and dermal administration, respectively.

4. Thin-layer chromatography: Nine TLC solvent systems were used to achieve separation of TNT and some potential metabolites. The R_f values of these compounds in some of the systems are shown in Table 22. Only solvents I, II, V, VII, and IX were found satisfactory, although no one solvent alone could completely resolve the available potential metabolites. The polar solvent system I was advantageous for separation of trinitrobenzoic acid and the diamino derivatives, which have low R_f values with the other solvents. TNT and the other potential metabolites showed better separation with the less polar solvents II, V, VII, and IX. Solvent systems I and VII were chosen for routine analysis. Later, solvent VII was replaced by solvent IX, which contains toluene instead of benzene.

a. Pilot studies: Pilot TLC studies were performed on ethyl acetate extracts from urine samples of rats, rabbits, and dogs treated orally with ^{14}C -TNT. The TLC profiles of these extracts are shown in Figures 6 (rats), 7 (rabbits) and 8 (dogs). The use of spray reagents coupled with the R_f values helped in the detection of certain metabolic products, e.g., the amines and hydroxylamines. Rat urine extracts demonstrated a complex metabolic pattern (Figure 6), and many of the metabolites were not identified. The presence of large amounts of diamines (more of 4,6-diamino and less of 2,6-diamino derivatives) and monoamines (2-amino and/or 4-amino) was confirmed with the positive reaction to Bratton-Marshall reagent. Areas on the plates other than those of the diamines and monoamines also responded positively to the reagent, but the identities of products located in these areas are not known. A feeble reaction with the TTC spray reagent at the R_f value of the 4-hydroxylamino suggested its presence in small amounts. The TLC profiles also suggested the presence of the alcohol, the acid, minute amounts of the azoxytoluene, and the parent compound (TNT).

The TLC profile of rabbit urine (Figure 7) indicated the presence of several metabolic products. Compared to rats, larger amounts of the two monoamines were present in rabbit urine. Their presence, as well as that of the diamines, was confirmed by a positive Bratton-Marshall reaction. The presence of the 4-hydroxylamines as well as small amounts of the 2-hydroxylamines was suggested by their position (R_f) on the TLC plates and by a positive red color after spraying with TTC reagent. As indicated earlier for the rat, the presence of the acid, the alcohol, and possibly minute quantities of the azoxytoluene and TNT was suggested by their positions on the TLC plates.

Dog urine (Figure 8) contained a large amount of the 4,6-diamine and less of the 2,6-diamine and the monoamine derivatives. The TLC profiles also suggested the presence of the acid, the alcohol, and minute amounts of the 4-hydroxylamine; the latter was indicated by the feeble reaction with TTC and Benedict's reagents.

Urine samples from rats, rabbits, and dogs were hydrolyzed with β -glucuronidase, and the mixtures were extracted with ethyl acetate. Figure 9 shows the TLC of the ethyl acetate extracts of rat urine incubated with either acetate buffer (Figure 9a) or the buffer plus β -glucuronidase (Figure 9b). The only difference between the urinary profiles of both extracts is the presence of a stronger peak at R_f value of about 0.19 after incubation with β -glucuronidase (Figure 9b, solvent VII). This peak corresponds to the 4,6-diamine reference metabolite. The profile of metabolites in hydrolyzed rabbit urine showed only slight quantitative differences from the nonhydrolyzed urine (Figure 10), whereas profiles in dog urine were the same after incubation with or without β -glucuronidase (Figure 11). Among species, major quantitative and probably qualitative differences occurred between urine profiles of rabbits on the one hand and dogs and rats on the other.

b. Definitive studies: TLC studies were performed on samples of raw urine, lyophilized urine, and extracts of urine and bile obtained from different species. The TLC plates were developed with either the polar solvent I or the less polar solvent IX. Radioactivity on the plates was processed by a computer program developed in our laboratory to obtain the profiles described below.

Figure 12 shows the TLC profiles obtained from raw urine of rats and mice treated orally, dermally, or intratracheally with ^{14}C -TNT. Urine of male rats showed the presence of several metabolites (Figure 12a), most of which were more polar than TNT, but a few which were less polar. Only small portions of the radioactivity developed with the less polar solvent IX. Urine from female rats (Figure 12b) behaved similarly except that larger amounts remained at the origin after developing with solvent I. Urine obtained from dermally treated male rats (Figures 12c and d) demonstrated the presence of some TNT and/or tetranitroazoxytoluene. Some TNT and/or tetranitroazoxytoluene were also noted in the 4-hr urine obtained after oral treatment of male rats with TNT (Figure 12e). The profiles of 24-hr (Figure 12a) and 4-hr (Figure 12e) urine after oral dosing were qualitatively similar. However, there appeared to be some differences between the metabolic profiles of 4-hr urine obtained from orally (Figure 12e) and intratracheally (Figure 12f) treated rats. Male mice treated orally or dermally showed similar profiles (Figures 12g and h), which were different, at least quantitatively, from profiles obtained from rat urine (Figure 12a). Peaks at the origin in solvent I were stronger in the profiles for mice. Identity of any of the metabolites cannot be suggested from these profiles since most of the radioactivity remained at the origin in the less polar solvent IX. However, the strong positive reactions which developed after spraying with Bratton-Marshall reagent indicated the presence of mono- and diamines among the metabolites excreted from rats and mice after oral and dermal treatment with TNT. No clear positive test was indicated after spraying the plates with a solution of TTC in sodium hydroxide, which detects the hydroxylamines.

The TLC profiles of lyophilized urine obtained from rats, mice, or rabbits are shown in Figure 13. Compared to raw urine, radioactivity in lyophilized urine demonstrated more tendency to migrate in both solvents. No radioactivity remained at the origin in solvent I. Urinary profiles of male rats (Figure 13a) and female rats (Figure 13b) treated orally were qualitatively similar. Urine from dermally treated rats showed some qualitative and quantitative differences. The presence of peaks corresponding to the parent compound (TNT) and the azoxytoluene was more apparent in dermally treated animals (Figures 13c and d). Urine obtained from male mice showed the presence of several metabolites (Figure 13e). A medium-sized peak between R_f 0.4 and 0.5 was composed of mostly the monoamines (as indicated by a positive reaction with Bratton-Marshall reagent), some hydroxylamines (a feeble red color after TTC), and probably some benzyl alcohol derivative. Urine from dermally treated mice showed stronger peaks corresponding to TNT and the azoxytoluene (Figure 13f). Rabbit urine obtained after both oral and dermal treatment (Figures 13g and h) demonstrated strong peaks corresponding to the R_f values of the diamines (4,6- and 2,6-diaminotoluenes) which gave positive reactions with Bratton-Marshall reagent. In the less polar solvent IX, only small amounts of the monoamines (4-amino and 2-amino-dinitrotoluene) were present. Small amounts of the 4-hydroxylamine derivative were also demonstrated in rabbit urine, but little, if any, of the azoxytoluene was present.

In another experiment, lyophilized urine from the four different species was processed by TLC, and the plates were cut into 0.5-cm zones (Figure 14). Migration of metabolites from the origin of the plates was demonstrated only with solvent I; most of the radioactivity remained at the origin with the less polar solvent IX. Urine from male rats (Figures 14a and c) seemed to have similar profiles to that from females (Figures 14b and d), whether dosing was oral or dermal. Urine from mice (Figures 14e and f) also showed similar profiles after oral and dermal treatment. The strong peak at R_f 0.34 of rabbit urine (Figure 14g) appears to be an artifact. Some differences were demonstrated in the profiles of urine obtained from dogs after oral and dermal dosing (Figures 14k and l). With solvent I, most radioactivity remained near the origin after oral administration (Figure 14k); after dermal application the radioactivity migrated readily (Figure 14l).

Figure 15 shows the TLC of the ethyl acetate-extractable material obtained from rat urine incubated with water, β -glucuronidase, or aryl sulfatase. With the more polar solvent I, all the activity as well as the reference metabolites migrated from the origin of the plates. Several metabolites could be demonstrated after developing with both solvents I and IX. The presence of the monoamines (4-amino and/or 2-amino derivatives) was clearly demonstrated (Figure 15a). Only small amounts of the diamino derivatives were detected. The presence of small amounts of the 4- and 2-hydroxylamines was suggested by the feeble reactions obtained after spraying with TTC or with Benedict's reagent. Although incubation with β -glucuronidase increased considerably the amounts of radioactivity extractable in ethyl acetate, there was no apparent change in TLC profiles with both solvents (Figure 15b). On the other hand, incubation with aryl sulfatase caused no change in the amounts of ethyl acetate-extractable radioactivity but seemed

to alter the metabolic profiles of both solvents (Figure 15c). There seemed to be a considerable increase in the peaks which corresponded to the diamino derivatives. TLC profiles of urine from female rats without or with enzyme hydrolysis were similar to those of male rats (Figures 15d, e, and f).

The TLC profiles of the ethyl acetate-extractable material from urine of male rats treated orally or dermally with ^{14}C -TNT are compared in Figure 16. After oral administration, these metabolites in urine migrated readily in solvent I (Figures 16a, e, and k). However, most of the metabolites were highly polar and remained at the origin in the less polar solvent IX. The presence of diamino and monoamino derivatives was readily demonstrated. Quantitative determination was not possible. Positive tests to TTC and Benedict's reagents suggested the presence of small amounts of the hydroxylamines. However, no azoxytoluene or TNT was demonstrated. From the TLC profiles, it appeared that the alcohol and the acid were present in small quantities. After hydrolysis with β -glucuronidase, only slight differences in the metabolic profiles were noted (Figures 16b, f, and l). Less radioactivity was recovered at the origin in solvent IX. Still, however, it constituted 65% of the activity applied on the plate. Urine obtained from rats treated dermally behaved similarly (Figures 16c, d, g, h, m, and n). Although the extracted radioactivity was higher after β -glucuronidase hydrolysis, the metabolic profiles did not change considerably, and a major portion of the activity still remained at the origins of the plates with solvent IX. When urine samples from different rats treated orally or dermally were compared, only slight quantitative differences seemed to be demonstrated. Some urine samples from dermally treated rats showed higher excretion of the parent compound, TNT.

The profiles of urinary metabolites obtained from female rats are shown in Figure 17. No apparent qualitative differences were noted between urine profiles of males and females. As noted in males, urine from dermally treated female rats showed slightly higher excretion of the parent compound, TNT.

The ethyl acetate extracts obtained from the 4-hr urine of male rats are shown in Figure 18. These rats were treated with ^{14}C -TNT orally or intratracheally. Bile was collected at the same time through a biliary cannula. Qualitatively, the metabolic profiles of these urine samples showed similarity to those of the 24-hr urine (Figure 16). Quantitatively, however, stronger peaks in the area of R_f 0.4 to 0.6 (solvent I) were present in the 4-hr urine. Hydrolysis with β -glucuronidase increased the extractable radioactivity but had no apparent effect on the pattern of metabolites (Figures 18a and b). Urine profiles from intratracheally treated rats showed some differences from urine profiles after oral dosing (Figures 18c and d). The presence of the diamines, the monoamines, and small amounts of the hydroxylamines was confirmed by the positive response to the chemical spray reagents.

The TLC profiles of 4-hr urine obtained from female rats treated orally or intratracheally are shown in Figure 19. As noted in urine of male rats, the metabolic pattern in solvent I was different from that obtained for the 24-hr urine samples (Figure 16). The TLC profiles obtained

from urine of orally treated animals (Figures 19a and b) were distinctly different from those of urine obtained after intratracheal dosing (Figures 19c and d). This difference was noted in both solvents I and IX.

Figure 20 shows the TLC profiles of the ethyl acetate-extractable material obtained from urine of male mice. Profiles in both solvents I and IX indicate that extensive metabolism of TNT occurred in mice. Hydrolysis with β -glucuronidase (Figures 20b, d, f, h, k, and l) did not cause any major changes in the metabolic patterns as compared with those without hydrolysis (Figures 20a, c, e, and g). Urine from dermally treated mice (Figures 20a, b, e, f, and l) was not qualitatively different from that obtained after oral dosing (Figures 20c, d, g, h, and k). Major differences between the urine from mice (Figure 20) and rats (Figure 16) were the presence of less polar metabolites at the origin of the TLC plates for mice, fewer diamino derivatives, but more of the monoamines, hydroxylamines, and probably the alcohol.

The urinary TLC profiles of the ethyl acetate extracts obtained from male rabbits treated with TNT are shown in Figure 21. As was noted for rat and mouse urine, rabbit urine without hydrolysis contained several metabolic products (Figures 21a, c, e, g, k, and m), many of which were highly polar. Quantitatively, rabbit urine contained larger amounts of the monoamines than did mouse urine. The 2,6- and/or 2,4-diamino derivatives were also present. Positive tests with TTC and Benedict's reagents confirmed the presence of the 4-hydroxylamine and to a lesser extent the 2-hydroxylamine. TLC profiles also suggested the presence of the alcohol and the acid, but this could not be confirmed. Of significance, TNT and the azoxytoluene were absent. In earlier studies, the hydroxylamines were found to decompose during the extraction processes leading to the azoxytoluene. The same metabolic profiles were obtained after incubation of rabbit urine with β -glucuronidase (Figures 21b, d, f, h, l, n, o, and p). In addition, profiles obtained from urine of dermally treated rabbits (Figures 21c, d, g, h, m, n, and p) were similar to those of orally treated rabbits (Figures 21a, b, e, f, k, l, and o), although smaller amounts of the acid seemed to be present in urine after dermal treatment.

The TLC profiles of urine samples obtained from male dogs treated orally or dermally are shown in Figure 22. The metabolic profiles of urine from dogs were qualitatively similar to those obtained from rats and mice. The presence of the monoamines, diamines, and hydroxylamines was confirmed by the positive reactions with Bratton-Marshall, TTC, and Benedict's reagents. No apparent differences were observed in the metabolic patterns of urine incubated with water or with β -glucuronidase. Urine from dermally treated dogs (Figures 21c, d, g, h, and l) appeared to contain less polar material at the origin of solvent IX and less acid as shown in solvent I.

The aqueous nonextractable material remaining after ethyl acetate extraction of rat, rabbit, and dog urine was evaporated under N_2 , then subjected to TLC analysis using the two solvent systems I and IX. Although some of the radioactivity migrated from the origin in solvent I, most remained at the origin in both solvents I and IX (Figure 23). Reaction with

Bratton-Marshall reagent suggested that only minute amounts of free amines were present in these aqueous fractions. Positive response to the reagent was detected in the areas corresponding to the diamines. These diamines are basic and are expected to remain in the aqueous phases after acidification with hydrochloric acid. Some, however, were detected in ethyl acetate extracts. No hydroxylamines were detected after spraying the plates with TTC or Benedict's reagents.

Figure 24 shows the TLC profiles of TNT metabolites in bile of rabbits and dogs. These bile samples were extracted with ethyl acetate after acidification with dilute hydrochloric acid. The extracted bile samples migrated readily with solvent I but to a lesser extent with solvent IX. The monoamines and, to a lesser extent, the diamines and hydroxylamines, were detected in rabbit bile (Figure 24a). Minute amounts of TNT were also present. In dog bile (Figure 24b), there was less radioactivity remaining at the origin of solvent I than in rabbit bile. The monoamines, diamines, and hydroxylamines were detected in dog bile obtained after oral treatment with TNT. The presence of the acid, alcohol, and TNT was suggested from their migration alongside the authentic metabolites. Hydrolysis with β -glucuronidase did not markedly alter the metabolic profiles of dog bile (Figure 24c). After dermal application of TNT, dog bile contained fewer polar metabolites and more of the parent compound, TNT (Figures 24d and e). The aqueous extracts obtained from dog bile (Figure 21f) contained highly polar metabolites. Except for the presence of some diamines (positive with Bratton-Marshall reagent), most of these metabolites were not identified.

c. Fractionated urinary metabolites: Urine samples from rats, mice, rabbits, and dogs were extracted with ether at different pH conditions in order to fractionate the urinary products into subgroups according to their neutral, acidic, or basic characteristics (see Figure 5a). The ether extracts (E₁-E₆) were evaporated and subjected to TLC analysis. A parallel experiment was performed in which TNT and nine potential metabolites were fractionated between the organic and aqueous phases. Recoveries of various compounds in the ether extracts were described in detail in an earlier section and are summarized as follows: E₁ contained large amounts of TNT, some of the monoamines, hydroxylamines, trinitrobenzyl alcohol, and azoxytoluene, and small amounts of the diamines. E₂ contained all the trinitrobenzoic acid, most of the monoamines, hydroxylamines, alcohol, and azoxytoluene, and some of the diamines and TNT. The basic fraction E₃ had most of the diamines and some of the monoamines. The E₂ fraction was subfractionated into E₄, which contained all the trinitrobenzoic acid, most of the hydroxylamines, trinitrobenzyl alcohol, and azoxytoluene, and some TNT; and E₅, which had most of the monoamines and some diamines. Subfraction E₆, derived from the E₄ fraction, contained most of the alcohol and azoxytoluene and some hydroxylamines.

The percentage of extractable radioactivity in different fractions of urines from different species and the TLC profiles of these different fractions are illustrated in Figures 25 through 40. Some of these profiles (e.g., E₃, E₅, and E₆) are simple and contain only a few major peaks, but others (e.g., E₁, E₂, and E₄) demonstrate complex patterns

of radioactive peaks. In almost every fraction, several metabolites of unknown identity were separated along with the anticipated products. Occasionally, a known metabolite was recovered in more than one fraction, and solubility characteristics seemed to have been altered in the presence of other metabolic products.

Figure 25 indicates the percentage of extractable radioactivity in different fractions of urine obtained from rats treated orally with TNT. The TLC profiles of the various fractions are shown in Figure 26. Fraction E₁ contained small amounts of the alcohol, monoamines, hydroxylamines, and diamines but may also have had some of the acid and probably the azoxytoluene and/or TNT. Most of the radioactivity was contained in highly polar material which did not migrate with the less polar solvent IX. Fraction E₂ was qualitatively similar to E₁, but it contained larger amounts of trinitrobenzoic acid and dinitrotoluenes. Also, major portions of the radioactivity remained at the origin when developed with solvent IX. In addition to the monoamines and diamines, fraction E₃ contained small amounts of hydroxylamines and the azoxytoluene but also other unidentified polar material. Fraction E₄ demonstrated a complex profile which contained large amounts of the trinitrobenzoic acid and lesser amounts of trinitrobenzyl alcohol, hydroxylamines, and TNT. In addition to some unidentified polar products, the basic fraction E₅ demonstrated large amounts of the monoamines, diamines, other unidentified amino derivatives (positive with Bratton-Marshall reagent), and small quantities of the azoxytoluene. E₆ contained large amounts of the alcohol and small quantities of azoxytoluene and hydroxylamines in addition to other unidentified metabolites. Most of the polar metabolites demonstrated in fraction E₄ were removed by sodium hydroxide and were absent from E₆.

The amount of extractable radioactivity from urine of dermally treated rats is illustrated in Figure 27. TLC profiles are shown in Figure 28. These profiles are similar to those obtained from urine of orally treated rats (Figure 26) with only a few exceptions. E₁ contained larger amounts of the parent compound, TNT, and/or the azoxytoluene. Fraction E₃ demonstrated the presence of less monoamines and more unidentified highly polar metabolites. On the other hand, more of the monoamines and fewer of the polar products were present in fraction E₅. E₆ contained appreciable amounts of TNT and/or the azoxytoluene.

Figure 29 summarizes the extractable radioactivity in different fractions of urine obtained from mice treated orally with ¹⁴C-TNT. The TLC profiles of the various fractions are shown in Figure 30. Fraction E₁ contained relatively large amounts of the monoamines and lesser quantities of the diamines, hydroxylamines, the alcohol, and the parent compound, TNT. Major differences between the profile of this fraction in mice (Figure 30) and rats (Figure 26) are the presence in mice of larger amounts of the monoamine and small amounts of the highly polar unidentified metabolites remaining at the origin after developing with solvent IX. E₂ demonstrated the presence of large amounts of trinitrobenzoic acid, some of the monoamines, diamines, hydroxylamines, and the alcohol. It also contained large quantities of unidentified polar products. The fraction E₃ contained mainly the

monoamines, some of the diamines, and some basic polar metabolites which reacted positively with Bratton-Marshall reagent. E₄ showed the presence of the acid, the alcohol, and some hydroxylamines and TNT. The E₅ fraction was spilled before analysis with TLC. E₆ contained mostly the alcohol, some hydroxylamines, and large amounts of TNT and/or the azoxytoluene. The latter is the likely possibility since a strong peak at this position was not demonstrated in fractions E₂ and E₄. It is probably formed from the hydroxylamines during the extraction of E₄ with sodium hydroxide.

The amounts of extractable radioactivity from urine of dermally treated mice are illustrated in Figure 31. TLC profiles are shown in Figure 32. These profiles are similar to those obtained from urine of orally treated mice (Figure 30), with the exception that E₁ and E₄ contained larger proportions of TNT and E₆ demonstrated stronger peaks corresponding to TNT and/or the azoxytoluene.

The extractable radioactivity in different fractions of urine obtained from rabbits treated orally with ¹⁴C-TNT is shown in Figure 33. The TLC profiles of the various fractions are illustrated in Figure 34. E₁ contained several metabolites which included varying amounts of the monoamines, hydroxylamines, alcohol, and some diamines. The absence of TNT and the azoxytoluene was demonstrated. A major portion of the radioactivity was contained in unidentified polar products. Fraction E₂ contained large amounts of the acid, some monoamines, hydroxylamines, diamines, and probably the alcohol. Only trace amounts of TNT and/or the azoxytoluene were present. E₃ contained mainly the monoamines and azoxytoluene and smaller quantities of the diamines. Large amounts of the acid, alcohol, and hydroxylamines and smaller quantities of the azoxytoluene were demonstrated in E₄. Fraction E₅ contained primarily the monoamines and diamines and other unidentified amino derivatives. The largest portion of E₆ is probably the alcohol. It also contained large amounts of the azoxytoluene and small proportions of the hydroxylamines. The azoxytoluene seemed to have been formed during the sodium hydroxide extraction of E₄.

The amounts of extractable radioactivity from urine of dermally treated rabbits are illustrated in Figure 35. TLC profiles are shown in Figure 36. The major difference between these profiles and those of urine obtained from orally treated rabbits (Figure 34) was in fraction E₁. Fraction E₁ from urine of dermally treated rabbits demonstrated increased amounts of the monoamines, hydroxylamines, alcohol, and azoxytoluene and a sharp decrease in the amounts of polar metabolites not migrating with solvent IX.

Figure 37 indicates the extractable radioactivity in different fractions of urine obtained from dogs treated orally with ¹⁴C-TNT. The TLC profiles of the various fractions are illustrated in Figure 38. Fraction E₁ contained several metabolic products including the monoamines, hydroxylamines, some diamines, and probably the alcohol. No TNT or azoxytoluene was demonstrated. The complex metabolic profile of E₂ contained the acid, monoamines, diamines, hydroxylamines, some TNT, and probably the trinitrobenzyl alcohol. E₃ contained mainly the monoamines, diamines, some TNT and/or azoxytoluene, and unidentified polar products. Fraction E₄ contained large amounts of the acid and some hydroxylamines, TNT, and probably the alcohol.

Large amounts of monoamines, the azoxytoluene and/or TNT were present in fraction E₅. Fraction E₆ contained large amounts of the alcohol and the parent compound, TNT, and small amounts of the hydroxylamines and the azoxytoluene. The latter appeared to be formed during the extraction of E₄ with sodium hydroxide.

The amounts of extractable radioactivity from urine of dermally treated dogs are illustrated in Figure 39. TLC profiles are shown in Figure 40. These profiles were similar to those obtained from urine of orally treated dogs (Figure 37) with only few exceptions. E₁ contained considerable amounts of TNT, which was absent from urine obtained after oral treatment. Fraction E₃ contained fewer monoamines and more of the diamines and unidentified polar metabolites. E₆ showed the presence of larger amounts of the parent compound, TNT, and the azoxytoluene.

5. Gas-liquid chromatography: Retention times of TNT and the available potential metabolites of TNT were determined as described in Section A, "Methods." The retention times for an isothermal (170°C) elution of TNT and potential metabolites are shown in Table 23. Attempts to achieve adequate separation of a mixture of TNT and the potential metabolites on either column were unsuccessful even when temperature programming was utilized.

6. High performance liquid chromatography: Different HPLC systems were tested for the separation of TNT and some potential metabolites. The first system used (system 1) was normal phase chromatography. It gave adequate separation of these compounds, but it was not adequate for the separation of more polar metabolites. Therefore, three other systems were examined which utilized counter-ion reverse phase chromatography. The retention times of TNT and some potential metabolites in this system are shown in Table 24. System 4 appeared to give good separation and the best defined peaks. This system was selected for the analysis of TNT and its metabolites in rat urine.

Figure 41 illustrates the chromatographic profile of raw urine obtained from rats treated orally with ¹⁴C-TNT. Some minor peaks were observed with retention times corresponding to those of TNT, the diamines, and the alcohol. However, most of the radioactivity in urine was eluted in adjacent fractions with similar retention times. Although some of these fractions have the same retention times as the 2-amino, 4-amino, and 2-hydroxylamino derivatives, confirmation of the presence of these metabolites was not possible. HPLC analysis was also performed on samples of rat urine hydrolyzed with β-glucuronidase. Although there were some apparent differences in the metabolic profiles after hydrolysis with β-glucuronidase (Figure 42), the identity of these metabolites was not confirmed. Better resolution of the metabolites in rat urine was obtained when smaller fractions of the eluted products were collected (Figure 43). However, the metabolic profile was also more complex. Since the use of HPLC offered no major advantage over TLC for the analysis of TNT profiles in urine of different species, its use was discontinued.

C. Discussion

1. Potential metabolites of TNT: Because of the presence of four functional groups on the TNT molecule, a variety of metabolic products could be formed. These may result from oxidation of the methyl group to alcohol, aldehyde, or acid; oxidation of the benzene nucleus to phenols; reduction of one or more of the nitro groups to hydroxylamino or amino compounds with the possibility of coupling of some of these metabolites; and conjugation of one or more of the resulting products (alcohols, acids, amines, hydroxylamines, etc.) to yield glucuronides, ethereal sulfates, substituted hippuric acid, or glutathione conjugates. Simultaneous oxidation and reduction followed by conjugation is also a possibility. These hypothetical pathways, which are shown in Figure 1, illustrate the complexity of the metabolic behavior of TNT. The problem of metabolite identification is complicated by the similar solubility characteristics possessed by these compounds of such closely related chemical structure.

Earlier studies by Voegtlin et al.⁴² and Dale⁵⁷ have suggested that the reduction products, 4-amino-2,6-dinitrotoluene and 2,6,2',6'-tetranitro-4,4'-azoxytoluene, are excreted in the urine of workers exposed to TNT. Reduction of a single nitro group of TNT was shown to occur also in rabbits, leading to the formation of 4-amino- and 6-amino-dinitrotoluenes.⁵⁶ Channon et al.⁵⁶ postulated that the first step in the reduction of the nitro group is the production of a hydroxylamine derivative. They isolated 4-hydroxylamino-2,6-dinitrotoluene as an aldoxime after reaction with benzaldehyde, but they failed to isolate its isomer, 2-hydroxylamino-4,6-dinitrotoluene. However, the isolation of the reduction product, 2-amino-4,6-dinitrotoluene, led to the conclusion that the 2-hydroxylamine is a step in its formation.

Because Wyon found the hydroxylamine derivative to be more toxic than the parent TNT, the isolation of hydroxylamine is of interest.⁵⁹ The hydroxylamine is a powerful methemoglobin producer *in vitro*, while TNT itself is only a weak producer of methemoglobin.⁵⁹ In addition, the formation of hydroxylamines is implicated in the carcinogenic responses induced by several carcinogenic amino and nitro compounds.⁶⁰ In Channon et al. studies, only 1% of the administered TNT dose was accounted for as hydroxylamine.⁵⁶ This, however, seems to be less than the actual amount present because of the great ease of conversion to the azoxy derivative.

Oxidation of TNT may result in the formation of alcohol or acid. These oxidation processes are hypothetical and are based on some indirect evidence obtained from some early studies by Channon et al.⁵⁶ Rabbits excreted 48% of the administered TNT dose as glucuronides, which were believed to arise from oxidation products of TNT such as trinitrobenzyl alcohol. The possibility of glucuronide conjugation with the amino or hydroxylamino derivatives was not considered. Also, the suggestion by Lemberg and Callaghan⁵⁵ that nitrophenylenediamine is excreted in rat urine indicates that this oxidative pathway may be operative. Williams⁶¹ suggested that the loss of the methyl group could probably occur by oxidation of TNT to the alcohol, then the acid, followed by decarboxylation and reduction of the nitro group.

Amino-nitrocresol is another oxidation product whose presence in rat urine was suggested. The mechanism of its formation is not known.

In vitro experiments suggested that the liver is the major site for TNT biotransformation.⁶² Studies using liver, muscle, and heart preparations showed that TNT was reduced by liver homogenates to 4-amino-2,6-dinitrotoluene. The rate of reduction was more rapid under anaerobic conditions. TNT metabolism occurred in a system containing reduced nicotinamide adenine dinucleotide (NADH) and a purified flavoprotein. It was also suggested that TNT was reduced to hydroxylamines by xanthine oxidase.

2. Extraction procedures: Since the beginning of this century, extensive work has been carried out to isolate and identify TNT metabolites in animals,⁵⁶⁻⁵⁸ and humans.^{55,57} Only limited success was achieved because of the difficulties encountered during the isolation procedures. Low recovery was encountered when urine samples were extracted with ether. It was found⁵⁶ that ether extracted little TNT-derived material from urine until it was acidified. Even after acidification, no more than 15% of the dose administered to rabbits was excreted as compounds soluble in ether. In the present study, the use of ether under mildly acidic conditions resulted in higher recoveries. This was further increased by extracting the urine with ethyl acetate under the same acidic conditions. The use of strong acid or base was avoided since this would undoubtedly cause alterations of the metabolites during the extraction process. 2,6,2',6'-Tetra-nitro-4,4'-azoxytoluene, which was reported as one of the TNT metabolites in rabbit and human urine,⁵⁷ was found later to be an artifact that was formed from the 4-hydroxylamine under the conditions of the isolation procedure. This azoxytoluene was shown to be absent from freshly voided urine of rabbits given TNT.⁵⁶ Alterations of TNT metabolites could also occur during storage. In our laboratory, the trinitrobenzyl alcohol and the trinitrobenzoic acid, two potential metabolites of TNT, were shown by TLC analysis to decompose to several products when stored in methanolic solutions.

Several methods were used in the present study to separate the metabolic products of TNT from urine and bile. Attempts to purify urine samples by Amberlite resin were not successful. Direct analysis of the raw or lyophilized urine was not successful, and separation of the highly polar and complex mixture of metabolic products proved difficult even with HPLC analysis. A more useful approach was the extraction of metabolic products into organic solvents. Acidification of the urine before extraction proved essential. Since these extracts still demonstrated complex metabolic profiles, a method was developed to fractionate the radioactivity in urine samples into subgroups according to their solubilities in the ether or aqueous extracts under different pH conditions. A mixture of TNT and nine potential metabolites was processed similarly and fractionated according to the neutral, acidic, or basic characteristics of each compound. Although this fractionation technique was successful when used with this mixture, it showed only limited success when urine samples were processed similarly. In almost every fraction, several metabolites of unknown identity were separated along with the anticipated products. Occasionally, a known metabolite was recovered in more than one fraction, and solubility characteristics seemed to have been altered in the presence of other metabolic products in the urine samples.

3. Separation procedures: Analysis of TNT metabolic profiles in urine and occasionally in bile was carried out by TLC. The use of GLC was discontinued since it was not possible to achieve a good separation of TNT and some potential metabolites. In view of the demonstrated high polarity of the excretory products, HPLC analysis was attempted. It offered no major advantage, however, over the use of TLC in this study since the major portion of the radioactivity excreted in urine was eluted in adjacent fractions. Although a good separation of synthetic mixtures was achieved by HPLC, poor separation occurred when urine samples were processed by this method. Studies to analyze the metabolic profiles of TNT in different species and after different routes of administration were, therefore, continued with TLC using two solvent systems with different polarity. TLC analysis had been useful in comparing the metabolic patterns of TNT metabolites. However, it required reference standards of potential metabolites for comparison, and many of these were not available commercially and could not be prepared in pure form in the MRI laboratories. Urine and bile contained large numbers of metabolic products. Attempts to isolate some of these metabolites in pure form by preparative TLC met with only limited success. The metabolites were assigned tentative identification based on comparing R_f values with those of some potential metabolites that were available and based on their solubility characteristics and reactions with certain specific chemical reagents. Because of the complexity of metabolic profiles, quantitative determinations were not possible.

4. Metabolic profiles: Early studies have suggested that urine from TNT workers contained the same metabolites reported in rabbit urine, namely 4-hydroxylamino-2,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and 2-amino-4,6-dinitrotoluene.⁵⁶ Rat urine contained, in addition to the monoamines, 2,4-diamino-6-nitrotoluene and probably 5-nitrophenylenediamine.⁵⁵ In the urine of dogs which received TNT orally, Snyder⁵⁸ was unable to demonstrate the presence of TNT, its oxidation products (alcohol, aldehyde, or acid), or its reduction products (diamino and triaminotoluenes). In the present studies, TNT profiles indicated that extensive biotransformation of TNT occurred in all species examined.

a. Rats: Metabolic profiles of rat urine demonstrated the presence of appreciable amounts of amino products (positive with Bratton-Marshall), some of which were not identified. Large quantities of diamines (more of 4,6-diamino derivative) and monoamines (the 4-amino and/or 2-amino) were present. Rat urine also contained small amounts of the 4-hydroxylamine and, to a lesser extent, the 2-hydroxylamine. Their presence was only demonstrated after ethyl acetate and/or ether extractions. Positive reactions with Benedict's reagent and the more specific triphenyltetrazolium chloride reagent indicated the presence of both hydroxylamines at the R_f positions of the corresponding standards.

The presence of appreciable amounts of trinitrobenzyl alcohol and trinitrobenzoic acid in rat urine was suggested by their R_f values. The latter compound was also indicated by its solubility behavior during the fractionation of urine. The absolute identity of both compounds, however, could not be confirmed.

Small amounts of azoxytoluene were detected in the TLC profiles of fractionated rat urine. The azoxytoluene was also found in urine of mice, dogs, and, to a greater extent, rabbits, mostly in the E₆ fraction after sodium hydroxide extraction of E₄. The azoxytoluene was probably formed from the hydroxylamines during the extraction process in the presence of alkalis.

Metabolic profiles of urine from male and female rats showed no significant differences. The urine collected from bile duct-cannulated rats showed metabolic profiles which differed, at least quantitatively, from that of the urine collected from noncannulated rats. It contained more polar metabolites and had more TNT and probably the azoxy compound. A quantitative difference was noted in the metabolic profiles of urines collected from orally versus intratracheally treated rats. On the other hand, the differences between urine collected from orally and dermally treated rats were minimal; more TNT was eliminated after dermal application.

Metabolism of TNT by the intestine or intestinal microflora was not examined in the present study. Others have demonstrated that the nitro group is highly susceptible to reduction by intestinal microflora.⁷⁵ From the present studies, it appears that TNT reduction occurred primarily in the liver. Bile and urine collected from biliary cannulated rats contained large amounts of reduced TNT metabolites, as indicated by the R_f values and the positive reactions with Bratton-Marshall, triphenyltetrazolium chloride and Benedict's reagents. This, however, does not rule out further intestinal metabolism occurring after excretion of the metabolic products through bile.

The nature of the red pigment excreted after TNT intake was examined by Channon et al.⁵⁶ They suggested that this pigment, which did not appear to account for a significant amount of metabolites, might be a partial reduction product of 2,4,6-trinitrobenzyl alcohol. They also suggested that the red pigment might be a salt of TNT or one of its metabolites since it decolorized on acidification with mineral acids. In the present study, rat urine was bright red in color even though urine is slightly acidic. On the other hand, rabbit urine is alkaline, but the red color was not apparent.

b. Mice: Mice also excreted monoamines, diamines, and small amounts of the hydroxylamines. The presence of azoxytoluene was demonstrated only after fractionation of urine samples under acidic and basic conditions. Considerable amounts of the benzyl alcohol and the acid seemed to be present. Metabolic profiles of urine from orally and dermally treated mice showed no major differences except for the presence of larger quantities of TNT in urine of dermally treated mice. Compared to rats, urine of mice contained lesser amounts of polar metabolites and diamines. Quantities of monoamines and hydroxylamines in urine of mice seemed to be larger than those in rats.

c. Rabbits: The metabolic profiles of rabbit urine demonstrated the presence of larger amounts of monoamines. The 4,6-diamine and, to a lesser extent, the 2,6-diamine were also present. The failure of earlier investigations to demonstrate the presence of diamines in rabbit urine

was probably due to the strong acid conditions used.⁵⁶ As noted in early studies, the 4-hydroxylamine was present in appreciable quantities in rabbit urine. The presence of smaller amounts of the 2-hydroxylamine was also suggested by comparison with the R_f value and behavior of an authentic sample. Urinary profiles of rabbit urine seem to contain trinitrobenzyl alcohol and trinitrobenzoic acid, as indicated by their R_f positions. With the mild extraction procedure used in this study, no azoxytoluene was demonstrated. However, after fractionation with ether in the presence of alkali, TLC peaks corresponding to azoxytoluene were found. Of significance was the absence of TNT from the urinary profiles of rabbits. After hydrolysis with β -glucuronidase, the urinary profiles remained the same. Urine obtained from dermally treated rabbits showed a sharp decrease in polar metabolites, including acid, and some increases in monoamines, hydroxylamines, and the azoxytoluene.

d. Dogs: The metabolic profiles of dog urine contained large amounts of the 4,6-diamines and the 2,6-diamines. Appreciable quantities of the monoamines (2- or 4-substituted) and probably the alcohol and acid were also present. The presence of small amounts of the 4-hydroxylamine and, to a lesser extent, the 2-hydroxylamine was indicated by comparing their migration and chemical behavior with authentic samples. The minute amounts of azoxytoluene present seemed to be formed during the extraction process. As shown in the urine of other species, β -glucuronidase hydrolysis caused no apparent differences in metabolic profiles. Urine obtained after dermal application contained smaller amounts of polar metabolites and larger amounts of the parent compound TNT as compared to urine from orally treated dogs.

5. Metabolite conjugates: Glucuronide conjugation appears to play an important role in the metabolism of TNT. Other conjugates and probably inorganic salts may also be present. Channon et al.⁵⁶ found that, even after acidification of rabbit urine, no more than 15% of the administered dose was excreted as compounds soluble in ether. They suggested that the ether extracts contained metabolites excreted in an unconjugated form and possibly small amounts of acetylated amino derivatives. The remainder of the doses administered were probably eliminated as conjugates, e.g., glucuronides and sulfates. The excretion of compounds in combination with glucuronic acid was suggested based on an increase in glucuronides in urine after TNT dosing. The present study confirms these earlier findings. Based on the increase in extractable radioactivity after hydrolysis with β -glucuronidase, major portions of TNT metabolites were excreted as glucuronide conjugates. The amounts of glucuronides varied among species. The least amounts occurred in urine of mice. Urine from dermally treated animals contained lesser amounts of glucuronide conjugates than did urine from orally treated animals. Amounts of glucuronide in urine from bile duct-cannulated rats were less than amounts from noncannulated rats. Bile contained large amounts of glucuronide conjugates; most compounds of low molecular weight, e.g., TNT metabolites, are excreted in bile only after conjugation with glucuronic acid or glutathione.

Although the extractable radioactivity increased considerably after hydrolysis with β -glucuronidase, major changes in the metabolic profiles after hydrolysis were not apparent. The only notable changes were increased

amounts of diamino metabolites in urine of some species, e.g., rat. On the other hand, some notable changes were demonstrated in the TLC profiles of rat urine after incubation with aryl sulfatase. Considerable increases in polar metabolites including the diamines occurred. However, there were no increases in the extractable radioactivity. This was taken as an indication of the absence of sulfate conjugates. Early studies have demonstrated no rise in ethereal sulfate excretion after administration of TNT to rabbits.⁵⁶

VII. CONCLUSIONS AND RECOMMENDATIONS

The present studies indicate that TNT administered orally, dermally, or intratracheally was readily absorbed, distributed, metabolized, and excreted in urine and to a lesser extent in feces. Absorption by the dermal route was slower than by the oral or intratracheal routes. Species differences in dermal absorption were found; the highest absorption occurred in rabbits, followed by mice, rats and dogs. TNT was more rapidly absorbed after intratracheal instillation than after oral or dermal administration. Biliary excretion and enterohepatic circulation appeared to play an important role in the disposition and metabolism of TNT.

TNT was metabolized extensively in all species examined, whether treatment was oral, dermal, or intratracheal. Most of the metabolic products were highly polar with very low extractability in organic solvents. Large portions of these products were conjugated with glucuronic acid, but no conjugation with sulfuric acid was detected. Other conjugates or inorganic salts of TNT metabolites were probably present. Most of the metabolic products were reduction derivatives, including the hydroxylamines, the monoaminodinitro and the diaminomononitro derivatives. The benzyl alcohol and the acid seemed to be present in medium quantities, but this was not confirmed. The parent compound, TNT, was demonstrated in the urine of some species but only in minute quantities. The mild extraction procedures used in the present study minimized the alterations of the hydroxylamines to the azoxytoluene, but the latter was present, especially after fractionation of the urinary products in the presence of NaOH. Other products of TNT metabolism were not identified due to lack of authentic standards for comparison.

Rabbit urine showed a unique metabolic profile which differed quantitatively, and probably qualitatively, from the metabolic profile of rats, mice, and dogs. The presence of larger quantities of the hydroxylamines and monoamines in rabbit urine was demonstrated. Rabbit urine also contained either or both of the diamines found in the urine of other species. The metabolic profiles of urine from rats, mice, and dogs also differed quantitatively. Even within species, some quantitative differences were demonstrated between individual animals. Major quantitative differences were demonstrated in the urinary profiles of orally versus intratracheally treated rats. On the other hand, the differences between urine profiles obtained from orally and dermally treated animals were minimal; larger amounts of the parent compound, TNT, were eliminated after dermal application. Although the extractable radioactivity increased considerably after β -glucuronidase hydrolysis of urine from different species following different routes of administration, major changes in the metabolic profiles were not apparent.

The results of the present study provide some data relevant to the selection of species and routes of exposure for any subsequent chronic toxicity studies. Based on urinary excretion patterns reported herein in comparison to earlier published information on humans, the present results would suggest that the rabbit may approximate humans more closely than do mice, rats, or dogs. The rabbit certainly excretes higher levels of at

least one of the potentially more active metabolites, hydroxylamine, which might enhance any potential carcinogenic responses. The rabbit, however, is not an animal commonly used in carcinogenic bioassays and certainly is not recommended herein for that purpose. The laboratory rat historically has been used extensively in carcinogenesis studies; the metabolic profile of TNT in rats is qualitatively similar to rabbits and also to humans. As such, the rat remains the most appropriate animal model for chronic studies. Mice could also be used for carcinogenic studies, but the only apparent advantage would be in the use of larger numbers of animals. Dogs would not be suitable for carcinogenic studies if for no other reason than the time interval (8 to 10 years) that might be required to undertake a study of this nature.

Human exposure to TNT for the most part is probably through the dermal and inhalation routes. Dermal exposure in humans can probably be simulated by oral exposure since the metabolic profiles in the experimental animals were qualitatively similar following oral and dermal exposures. The major issue would be to adjust dose levels or at least to interpret experimental results as a function of absorption since the present data demonstrated less absorption of TNT following dermal application to rodents. Additional metabolism studies would be warranted to better define relative absorption following oral and dermal exposures.

The question of simulation of inhalation exposure using oral administration could not be completely resolved in the present studies. Failure to produce adequate dispersions or aerosols for inhalation exposures negates any direct resolution of the problem. Metabolic data, however, obtained following intratracheal instillation demonstrated quantitative differences in urinary profiles, hence metabolism of the compound. Moreover, absorption, distribution, and elimination were more rapid following intratracheal instillation. On this basis, use of oral exposures to simulate inhalation exposures would not appear to be appropriate. However, because of the apparent problems associated with the generation of aerosols for inhalation, oral exposures may be necessary.

The need for further research is obvious. Additional efforts should be directed to developing techniques to produce aerosols or particulate dispersions appropriate for inhalation studies. If successful, additional metabolic studies would be required to determine absorption, distribution, metabolism, and excretion of TNT following inhalation exposure. If not successful and oral exposures are used for chronic studies, additional metabolism studies would be warranted to better correlate TNT disposition and metabolism following oral exposure and intratracheal instillation on the assumption that this route would simulate absorption after inhalation exposure.

REFERENCES

1. Kirk-Othmer, α or 2,4,6-Trinitrotoluene. Kirk-Othmer Encycl. Chem. Technol., 2nd ed., 8, 611-616 (1965).
2. Buck, C. R., and S. E. Wilson. Occupational health effects of selected explosives (TNT, RDX). U.S. Army Environmental Hygiene Agency Report No. 32-049-75/76, 107 pp (1975).
3. American Industrial Hygiene Association. Hygienic Guide Series: 2,4,6-trinitrotoluene. J. Am. Ind. Hyg. Assoc., 25, 516-519 (1964).
4. Fairhill, L. T. Industrial Toxicology. Williams and Wilkins, Baltimore, pp. 459-462, 224-226, 446-449 (1949).
5. Daniel, N. B. Liver damage at an ordnance plant--incidence and prevention. Ind. Med., 23, 409-410 (1954).
6. Evans, R. M. TNT jaundice. Lancet, 1, 552-554 (1941).
7. Hathaway, J. A. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. J. Occup. Med., 19, 341-345 (1977).
8. Panton, P. N. The effect of trinitrotoluene upon the blood. Lancet, 2, 77-82 (1917).
9. Hart, W. L., E. B. Ley, V. D. Scroggie, E. A. Johnson, and J. H. Eddy. A report of four cases of aplastic anemia occurring among munitions workers. Ind. Med., 13, 896-899 (1944).
10. Crawford, M. A. D. Aplastic anemia due to trinitrotoluene intoxication. Br. Med. J., 2, 430-437 (1954).
11. Pinto, S. S., and M. Bowditch. Industrial hygiene. N. Engl. J. Med., 225, 949-952 (1941).
12. Sievers, R. E., R. L. Stump, and A. R. Monaco. Aplastic anemia following exposure to trinitrotoluene. Report of three cases. Occup. Med., 1, 351-362 (1946).
13. Soboleva, L. P. [State of the myocardium during chronic trinitrotoluene intoxication.] Gig. Tr. Prof. Zabol., 13, 47-48 (1969); Chem. Abstr., 72, 93149e (1970).
14. Vychub, V. N. [Capillary permeability and strength in workers suffering from trinitrotoluene poisoning.] Gig. Tr. Prof. Zabol., 14, 54-56 (1970); Biol. Abstr., 52, 46309 (1971).

15. Kaganov, A. L., and E. I. Epshtein. [The problem of occupationally induced chemotoxic nephropathies.] Gig. Tr. Prof. Zabol., 13, 31-35 (1969).
16. Kennedy, A. M., and J. Ingham. Prophyrinuria in trinitrotoluene poisoning. Br. Med. J., 1, 490-492 (1942).
17. Kleiner, A. I. [Influence of trinitrotoluene on pancreatic exocrine function.] Ter. Arkh., 38, 52-55 (1966); Chem. Abstr., 65, 7884c (1966).
18. Krol, D. S., and V. P. Kolevathkh. [Ring cataract with chronic trinitrotoluene (TNT) poisoning.] Oftalmol. Zh., 20, 180-183 (1965); Biol. Abstr., 47, 47719 (1966).
19. Pen'kov, M. A. [Changes in the eyes in underground explosives handlers.] Gig. Tr. Prof. Zabol., 9, 52-53 (1965); Biol. Abstr., 47, 103651 (1966).
20. Aiello, G. [TNT intoxications and ocular diseases.] Ann. Oftalmol. Clin. Ocul., 72, 17-21 (1946).
21. Logan, I. M., Z. M. Skripnichenko, and E. T. Tkachenko. [Trinitrotoluene (TNT) cataract in miners, its diagnosis and prevention.] Oftalmol. Zh., 25, 579-584 (1970).
22. Glezerov, S. Ya. [Cataract after intoxication with nitrokraskoi (a nitro dye).] Vestn. Oftalmol., 69, 46-49 (1956); Chem. Abstr., 51, 3835a (1957).
23. Tyukina, G. A. [Some characteristics of the clinical aspects of trinitrotoluene-induced cataracts.] Vestn. Oftalmol., 81, 43-47 (1967).
24. Hunter, D. The Diseases of Occupations. Little, Brown and Company, Boston, pp. 470-480 (1955).
25. Minot, G. R. Blood examinations of trinitrotoluene workers. J. Ind. Hyg., 1, 301-319 (1919).
26. Brunetti, P., and F. Grignani. [Enzyme pathogenesis of acute hemolytic anemia due to trinitrotoluene.] Lav. Um., 11, 350-358 (1959).
27. Djerassi, L. S., and L. Vitany. Haemolytic episode in G6PD deficient workers exposed to TNT. Br. J. Ind. Med., 32, 54-58 (1975).
28. Hassman, P. [Determination of 2,6-dinitro-4-aminotoluene in the urine of persons exposed to trinitrotoluene.] Prac. Lek., 23, 312-314 (1971); Chem. Abstr., 76, 56228c (1972).
29. Schepers, G. W. H. Lung tumors of primates and rodents: Part II. Ind. Med. Surg., 40(2), 23-31 (1971).
30. Hamilton, A. Industrial poisons encountered in the manufacture of explosives. J. Am. Med. Assoc., 68, 1445-1451 (1917).

31. McConnell, W. J., and R. H. Flinn. Summary of twenty-two trinitrotoluene fatalities in World War II. J. Ind. Hyg., 28, 76-86 (1946).
32. Fischer. [Fatal industrial poisonings through trinitrotoluene and tetranitromethane.] Zentralbl. Gewerbehyg. Unfallverhuet., 5, 205-217 (1917); Chem. Abstr., 13, 791 (1919).
33. Teisinger, J. [Chronic TNT action and influence of alcohol on its transformation in the body.] Arch. Gewerbepathol. Gewerbehyg., 4, 491-499 (1933); Chem. Abstr., 27, 3751 (1933).
34. Eddy, J. H., Jr. Aplastic anemia following trinitrotoluene exposure. J. Am. Med. Assoc., 125, 1169-1172 (1944).
35. Zakharova, A. I., and I. K. Manoilova. [Clinical picture in chronic trinitrotoluene poisoning.] Gig. Tr. Prof. Zabol., 15, 28-32 (1971).
36. Bizzarri, M. [Hematological studies in experimental poisoning with trinitrotoluene.] Folia Med. Naples, 25, 801-828 (1939); Chem. Abstr., 36, 35503 (1942).
37. Fimiani, R. [Coproporphyrinuria in experimental intoxication due to trinitrotoluene.] Folia Med. Naples, 32, 617-624 (1949); Chem. Abstr., 44, 3151f (1950).
38. Ambrosio, L. [Ketonemic and ketonuric modifications in TNT experimental intoxication.] Folia Med. Naples, 34, 212-223 (1951); Chem. Abstr., 46, 4114c (1952).
39. Pecora, L. [Protein metabolism in experimental poisoning by TNT.] Folia Med. Naples, 32, 487-480 (1949); Chem. Abstr., 44, 2651c (1950).
40. Geshev, G., and V. Kincheva. [Chromosomal changes in rats after trinitrotoluene treatment.] Probl. Akush. Ginekol., 2, 111-114 (1974).
41. Won, W. D., L. H. DiSalvo, and J. Ng. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. Appl. Environ. Microbiol., 31(4), 576-580 (1976).
42. Voegtlin, C., C. W. Hooper and J. M. Johnson. Trinitrotoluene poisoning. Pub. Health. Rep., 34, 1307-11 (1919).
43. Gring, D. M. Biological effects of trinitrotoluene (TNT). Doctoral dissertation, Department of Zoology, Indiana University, 1-6, 81-89. Full Text Microfilm No. 72-6782-1-536905, Desk of R. Kahn (1971); Chem. Abstr., 76, 149640k (1972).
44. Saz, A. K., and R. B. Slie. The inhibition of organic nitroreductase by aureomycin in cell-free extracts. II. Cofactor requirements for the nitro reductase enzyme complex. Arch. Biochem. Biophys., 5, 5-16 (1954).

45. Enzinger, R. M. Special study of the effect of alpha TNT on microbiological systems and the determination of the biodegradability of alpha TNT. U.S. Army Environmental Hygiene Agency Sanitary Engineering Special Study No. 24-017-70/71, 24 pp. (1971).
46. Putnam, T. J., and W. Herman. A study of fifty workers in trinitrotoluene. J. Ind. Hyg., 1, 238-245 (1919).
47. Voegtlin, C., K. W. Hooper, and J. M. Johnson. Trinitrotoluene poisoning--its nature, diagnosis and prevention. J. Ind. Hyg., 3, 239-254 (1921).
48. Von Oettingen, W. F., D. D. Donahue, R. K. Snyder, T. R. Sweeney, and A. R. Monaco. V. Toxicity of TNT for dogs with daily insufflation of TNT dust. Public Health Bull., 285, 20-36 (1944).
49. Von Oettingen, W. F., The aromatic amino and nitro compounds, their toxicity and potential dangers: a review of the literature. U.S. Public Health Service, Public Health Bull., 271 (1941).
50. Horecker, B. L., and R. K. Snyder. Effect of ingestion of small quantities of TNT to humans. Public Health Bull., IX, 285, 50-52 (1944).
51. Miller, K. C. Toxicity and adverse effects of trinitrotoluene (TNT): A partially annotated bibliography. Toxicology Information Response Center, Oak Ridge National Laboratory ORNL-TIRC-73-15, 14 (1973).
52. Neal, P. A., W. F. von Oettingen, and T. R. Sweeney. Absorption of TNT through the intact skin of swine. Public Health Bull., X, 285, 53-54 (1944).
53. Haythorn, S. R. Experimental trinitrotoluene poisoning. J. Ind. Hyg., 2, 298-318 (1920).
54. Neal, P. A., W. F. von Oettingen, and R. K. Snyder. Absorption of TNT through the intact skin of human subjects. Public Health Bull., XI, 285, 55 (1944).
55. Lemburg, R., and J. P. Callaghan. Metabolism of aromatic nitro compounds; I. Estimation of diazotisable amines in rats' and human urine after intake of 2,4,6-trinitrotoluene; II. Excretion of diazotisable amines in the urine after intake of TNT and a reduction product of TNT; III. Isolation of reduction products of 2,4,6-trinitrotoluene from the urine of rats and from human urine. Austral. J. Expt. Biol. Med. Sci., 23, 1-20 (1945).
56. Channon, H. J., G. T. Mills, and R. T. Williams. The metabolism of 2,4,6-trinitrotoluene (α -TNT). Biochem. J., 38, 70-85 (1944).
57. Dale, H. H. The fate of TNT in the animal body. Gr. Brit. Med. Res. Council, Spec. Rep., Series No. 58 (1921).

58. Snyder, R. K. Metabolites of 2,4,6-trinitrotoluene (TNT) excreted in the urine of dogs. J. Ind. Hyg. Toxicol., 28, 59-75 (1946).
59. Wyon, G. A. Experiments on the toxic effects of trinitrotoluene in animals. Gr. Brit. Med. Res. Council, Spec. Rep., Series No. 58, 32-48 (1921).
60. Weisburger, J. H., and E. K. Weisburger. Biochemical formation and pharmacological, toxicological, and pathological properties of hydroxylamines and hydroxamic acids. Pharmacol. Rev., 25, 1-66 (1973).
61. Williams, R. T. Detoxification mechanisms: the metabolism and detoxification of drugs, toxic substances, and other organic compounds. John Wiley, New York, 2nd ed., pp. 410-413, pp. 417-420, pp. 425-427 (1959).
62. Bueding, E., and N. Jolliffe. Metabolism of trinitrotoluene (TNT) in vitro. J. Pharm. Exp. Ther., 88, 300-312 (1946).
63. Elvove, E. The detection and estimation of small amounts of certain organic nitro compounds with special reference to the examination of the urine of TNT workers. J. Ind. Eng. Chem., 11, 860-864 (1919).
64. Ganguly, K. Halogenation of 2,4,6-trinitrotoluene. Berichte, 58B, 708-712 (1925).
65. Parkes, G. D., and A. C. Farthing. Derivatives of 2,4,6-trinitrotoluene: monoreduction of polynitro compounds. J. Chem. Soc., 1275-1278 (1948).
66. Saffiotti, U., F. Cefis, and L. H. Kolb. A method for the experimental induction of bronchogenic carcinoma. Cancer Res., 28, 104-124 (1968).
67. Feron, V. J. Respiratory tract tumors in hamsters after intratracheal instillations of benzo(a)pyrene alone and with furfural. Cancer Res., 32, 28-36 (1972).
68. Henry, M. C., C. D. Port, R. R. Bates, and D. G. Kaufman. Respiratory tract tumors in hamsters induced by benzo(a)pyrene. Cancer Res., 33, 1585-92 (1973).
69. El-hawari, A. M., and G. L. Plaa. Role of the enterohepatic circulation in the elimination of diphenylhydantoin in the rat. Drug Met. Dispos., 6, 59-69 (1978).
70. Mahin, D. T., and R. T. Lofkey. A simplified method of sample preparation for determination of tritium, carbon-14, or sulfur-35 in blood or tissue by liquid scintillation counting. Anal. Biochem., 16, 500-509 (1966).

71. Grindel, J. M., R. S. Rozman, D. M. Leah, N. A. Molek, and H. H. Gillum. The absorption, distribution, and excretion in mice of a quinoline-methanol antimalarial, 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-t-butylamino)propyl]quinoline phosphate. Drug Metab. Dispos., 4, 133-139 (1976).
72. Bratton, A. C., E. K. Marshall, Jr., D. Babbitt, and A. R. Hendrickson. A new coupling component for sulfanilamide determination. J. Biol. Chem., 128, 537-550 (1939).
73. Feigl, F., V. Anger, and R. E. Oesper. Spot Tests in Organic Analysis, Seventh Edition. Elsevier Publishing Company, New York, p. 300 (1966).
74. Coutts, R. T., and A. M. El-hawari. Cyclic hydroxylamines: A review of preparative methods and properties. Heterocycles, 2, 669-743 (1974).
75. Scheline, R. R. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol. Rev., 25, 451-523 (1973).

TABLE 1

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL
ADMINISTRATION OF ^{14}C -TNT (100 mg/kg) TO SPRAGUE-DAWLEY RATS^a

Tissue/Excretum	Males		Females	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	2.884 \pm 0.676	0.200 \pm 0.047	4.175 \pm 0.679	0.292 \pm 0.047
Liver	10.748 \pm 2.559	0.385 \pm 0.100	13.854 \pm 3.415	0.428 \pm 0.069
Kidneys	3.491 \pm 0.720	0.169 \pm 0.031	2.575 \pm 0.731	0.245 \pm 0.052
Lungs	0.299 \pm 0.080	0.019 \pm 0.006	0.378 \pm 0.117	0.038 \pm 0.010
Spleen	1.767 \pm 0.423	0.002 \pm 0.000	4.718 \pm 3.011	0.003 \pm 0.002
Brain ^c	0.152 \pm 0.067	0.008 \pm 0.003	0.221 \pm 0.064 ^d	0.016 \pm 0.003 ^d
Muscle	0.777 \pm 0.213	0.309 \pm 0.085	2.154 \pm 0.153 ^d	0.863 \pm 0.062 ^d
GI Tract plus contents	91.217 \pm 8.891	29.756 \pm 2.681	99.821 \pm 23.648	33.937 \pm 6.456 ^d
Feces		8.050 \pm 2.444		2.057 \pm 0.767 ^d
Urine		52.719 \pm 4.095		64.549 \pm 4.178 ^d
Recovery		91.624 \pm 6.633		102.430 \pm 8.787

^a Mean \pm SE of four rats per group.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Significantly different ($p < 0.05$) from males.

TABLE 2

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL
ADMINISTRATION OF ^{14}C -TNT (100 mg/kg) TO SWISS MICE^a

Tissue/Excretum	Males		Females	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	6.936 \pm 4.297	0.498 \pm 0.311	1.023 \pm 0.130 ^d	0.069 \pm 0.007 ^d
Liver	14.845 \pm 3.594	0.756 \pm 0.201	8.372 \pm 1.108 ^d	0.500 \pm 0.073 ^d
Kidneys	29.698 \pm 21.768	0.541 \pm 0.418	5.056 \pm 0.539 ^d	0.070 \pm 0.005
Lungs	9.504 \pm 3.239	0.057 \pm 0.018	6.383 \pm 1.464	0.034 \pm 0.009
Spleen	4.498 \pm 2.004	0.007 \pm 0.002	2.725 \pm 0.605	0.006 \pm 0.001
Brain ^c	1.751 \pm 0.595	0.026 \pm 0.008	0.932 \pm 0.186	0.017 \pm 0.003
Muscle	1.943 \pm 0.775	0.794 \pm 0.320	1.398 \pm 0.308	0.480 \pm 0.128
GI Tract plus contents	7.450 \pm 2.163	13.453 \pm 3.339	7.963 \pm 0.604	7.421 \pm 0.796 ^d
Feces		22.012 \pm 1.210		8.959 \pm 1.059 ^d
Urine		41.910 \pm 6.785		42.874 \pm 3.985
Recovery		80.058 \pm 5.224		60.435 \pm 2.599 ^d

^a Mean \pm SE of seven male or eight female mice.

^b Based on 7% body weight.

^c Based on 40% of body weight.

^d Significantly different ($p < 0.05$) from males.

TABLE 3

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL
ADMINISTRATION OF ^{14}C -TNT (5 mg/kg) TO NEW ZEALAND RABBITS^a

Tissue/Excretum	Males		Females	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	0.199 \pm 0.066	0.278 \pm 0.099	0.277 \pm 0.090	0.441 \pm 0.168
Liver	1.450 \pm 0.491	0.821 \pm 0.327	1.681 \pm 0.347	0.935 \pm 0.255
Kidneys	0.549 \pm 0.147	0.056 \pm 0.014	0.927 \pm 0.240	0.099 \pm 0.031
Lungs	1.688 \pm 0.665	0.146 \pm 0.057	3.828 \pm 2.952	0.293 \pm 0.230
Spleen	0.173 \pm 0.043	0.000 \pm 0.000	0.296 \pm 0.122	0.001 \pm 0.000
Brain ^c	0.091 \pm 0.012	0.004 \pm 0.000	0.127 \pm 0.049	0.006 \pm 0.002
Muscle	0.098 \pm 0.004	0.771 \pm 0.072	0.195 \pm 0.080	1.761 \pm 0.799
GI Tract plus contents	1.493 \pm 1.066	7.495 \pm 5.171	1.186 \pm 0.180	4.719 \pm 0.704
Feces		1.776 \pm 1.728		1.827 \pm 0.197
Urine		66.296 \pm 8.304		78.857 \pm 16.304
Recovery		77.645 \pm 2.683		88.940 \pm 18.259

^a Mean \pm SE of three rabbits per group.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

TABLE 4

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL
ADMINISTRATION OF ^{14}C -TNT (5 mg/kg) TO BEAGLE DOGS^a

Tissue/Excretum	Males		Females	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	0.979 \pm 0.142	1.383 \pm 0.192	1.397 \pm 0.181	1.956 \pm 0.256
Liver	4.041 \pm 1.017	2.176 \pm 0.516	2.642 \pm 0.191	1.786 \pm 0.332
Kidneys	1.098 \pm 0.113	0.101 \pm 0.007	1.572 \pm 0.154	0.162 \pm 0.012
Lungs	0.705 \pm 0.016	0.200 \pm 0.104	1.524 \pm 0.702	0.213 \pm 0.094
Spleen	0.961 \pm 0.064	0.183 \pm 0.009	1.304 \pm 0.183	0.208 \pm 0.059
Brain ^c	0.275 \pm 0.032	0.038 \pm 0.006	0.375 \pm 0.101	0.063 \pm 0.008
Muscle ^c	0.239 \pm 0.010	1.940 \pm 0.112	0.316 \pm 0.025	2.526 \pm 0.187
GI Tract plus contents	4.742 \pm 3.059	9.997 \pm 6.619	2.091 \pm 0.941	4.396 \pm 1.948 ^d
Feces		5.411 \pm 3.173		16.790 \pm 3.836 ^d
Urine		55.918 \pm 8.809		60.157 \pm 1.463
Recovery		77.350 \pm 5.485		88.259 \pm 3.629

^a Mean \pm SE of three dogs per group.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Significantly different ($p < 0.05$) from males.

TABLE 5

TISSUE-TO-BLOOD CONCENTRATION RATIOS IN RATS, MICE, RABBITS, AND DOGS
AT 24 HR FOLLOWING ORAL ADMINISTRATION OF ¹⁴C-TNT

Tissue	Rats		Mice		Rabbits		Dogs	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	3.7	3.3	3.0	8.1	7.3	6.0	4.1	1.9
Kidneys	1.2	0.6	4.3	4.9	2.8	3.3	1.0	1.1
Lungs	0.1	0.1	1.4	6.2	8.5	13.7	0.7	1.1
Spleen	0.6	1.1	0.7	2.6	0.9	1.1	1.0	0.9
Brain	0.1	0.1	0.3	0.9	0.5	0.5	0.3	0.3
Muscle	0.3	0.5	0.3	1.3	0.5	0.7	0.2	0.2
Blood (µg/ml)	1.0 (2.88)	1.0 (4.18)	1.0 (6.94)	1.0 (1.02)	1.0 (0.20)	1.0 (0.28)	1.0 (0.98)	1.0 (1.4)

TABLE 6

LEVELS OF RADIOACTIVITY IN BLOOD FOLLOWING ORAL OR DERMAL ADMINISTRATION
OF ^{14}C -TNT (50 mg/kg) TO RATS^a

Time After Treatment (hr)	Concentration ($\mu\text{g eq/ml}$)			
	Oral		Dermal	
	Males	Females	Males	Females
4	4.62 \pm 0.65	5.82 \pm 0.62	0.96 \pm 0.13 ^b	1.42 \pm 0.23 ^b
8	5.73 \pm 0.41	7.41 \pm 0.53	1.33 \pm 0.16 ^b	2.23 \pm 0.31 ^b
24	1.77 \pm 0.23	2.72 \pm 0.19	1.90 \pm 0.18	2.43 \pm 0.33

^a Mean \pm S.E. of three rats per treatment.

^b Significantly different ($p < 0.05$) from oral treatment.

TABLE 7

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO MALE SPRAGUE-DAWLEY RATS^a

Tissue/Excretum	Oral		Dermal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	1.77 \pm 0.22	0.25 \pm 0.03	1.44 \pm 0.19	0.23 \pm 0.03
Liver	7.29 \pm 0.48	0.45 \pm 0.03	2.81 \pm 0.31 ^e	0.15 \pm 0.01 ^e
Kidneys	5.80 \pm 108	0.26 \pm 0.09	3.06 \pm 0.42 ^e	0.05 \pm 0.006 ^e
Lungs	2.05 \pm 0.27	0.016 \pm 0.002	1.44 \pm 0.20	0.01 \pm 0.002
Spleen	1.01 \pm 0.28	0.003 \pm 0.001	0.57 \pm 0.16	0.002 \pm 0.000
Brain ^c	0.56 \pm 0.14	0.007 \pm 0.007	0.85 \pm 0.14	0.011 \pm 0.002
Muscle ^c	0.88 \pm 0.37	0.70 \pm 0.29	0.58 \pm 0.15	0.46 \pm 0.012
Fat	1.13 \pm 0.68	-	2.39 \pm 0.25 ^e	-
GI Tract plus contents	228.4 \pm 6.6	20.24 \pm 1.85	35 \pm 1.31	3.11 \pm 0.30 ^e
Feces		10.72 \pm 0.88		1.32 \pm 0.13 ^e
Urine		59.54 \pm 0.95		17.35 \pm 2.09 ^e
Recovery ^d		92.19 \pm 1.91		22.76 \pm 1.85 ^e

^a Mean \pm SE of three (oral) or six (dermal) rats.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat and skin (including site of application) are not included in the recovery estimates.

^e Significantly different ($p < 0.05$) from oral treatment.

TABLE 8

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO FEMALE SPRAGUE-DAWLEY RATS^a

Tissue/Excretum	Oral		Dermal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	2.27 \pm 0.60	0.34 \pm 0.11	2.06 \pm 0.26	0.29 \pm 0.03
Liver	5.53 \pm 0.76	0.33 \pm 0.05	3.06 \pm 0.33 ^e	0.17 \pm 0.01 ^e
Kidneys	4.45 \pm 0.43	0.066 \pm 0.009	4.01 \pm 0.35	0.06 \pm 0.005
Lungs	2.10 \pm 0.20	0.016 \pm 0.002	1.69 \pm 0.17	0.01 \pm 0.001
Spleen	0.98 \pm 0.18	0.003 \pm 0.001	0.53 \pm 0.08	0.002 \pm 0.000
Brain ^c	0.50 \pm 0.07	0.007 \pm 0.001	1.18 \pm 0.67	0.014 \pm 0.007
Muscle ^c	0.70 \pm 0.17	0.56 \pm 0.14	1.12 \pm 0.53 ^e	0.86 \pm 0.43
Fat	0.81 \pm 0.18	-	3.81 \pm 0.70 ^e	-
GI Tract				
plus contents	410.9 \pm 56.4	35.29 \pm 3.94	55.8 \pm 3.5 ^e	6.40 \pm 0.58 ^e
Feces		2.14 \pm 0.23		2.49 \pm 0.31 ^e
Urine		42.54 \pm 1.54		14.55 \pm 2.29 ^e
Recovery ^d		81.30 \pm 3.29		24.85 \pm 1.82 ^e

^a Mean \pm SE of three (oral) or six (dermal) rats.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat and skin (including site of application) are not included in the recovery estimates.

^e Significantly different ($p < 0.05$) from oral treatment.

TABLE 9

**TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO MALE SWISS MICE^a**

Tissue/Excretum	Oral		Dermal	
	Concentration ($\mu\text{g eq/g or ml}$)	Percent of Dose	Concentration ($\mu\text{g eq/g or ml}$)	Percent of Dose
Blood ^b	0.93 \pm 0.28	0.17 \pm 0.03	1.23 \pm 0.24	0.17 \pm 0.03
Liver	4.79 \pm 0.35	0.40 \pm 0.03	3.32 \pm 0.45 ^e	0.30 \pm 0.03 ^e
Kidneys	3.07 \pm 0.39	0.088 \pm 0.01	3.08 \pm 0.49	0.09 \pm 0.01
Lungs	1.58 \pm 0.23	0.015 \pm 0.002	1.36 \pm 0.20	0.014 \pm 0.001
Spleen	1.08 \pm 0.21	0.013 \pm 0.008	0.56 \pm 0.15	0.010 \pm 0.006
Brain ^c	0.42 \pm 0.07	0.011 \pm 0.002	0.67 \pm 0.19	0.017 \pm 0.004
Muscle ^c	0.49 \pm 0.10	0.385 \pm 0.083	0.75 \pm 0.12	0.610 \pm 0.088
Fat	0.71 \pm 0.15	-	3.25 \pm 1.51 ^e	-
GI Tract plus contents	50.31 \pm 3.84	10.19 \pm 0.75	17.03 \pm 1.18 ^e	3.61 \pm 0.25 ^e
Feces		24.07 \pm 0.83		14.17 \pm 1.31 ^e
Urine		59.05 \pm 5.32		22.68 \pm 2.44 ^e
Recovery ^d		94.39 \pm 2.16		41.69 \pm 2.53 ^e

^a Mean \pm SE of eight (oral) or six (dermal) mice.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat and skin (including site of application) are not included in the recovery estimates.

^e Significantly different from oral treatment ($p < 0.05$).

TABLE 10

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (5 mg/kg) TO MALE NEW ZEALAND RABBITS^a

Tissue/Excretum	Oral		Dermal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	0.260 \pm 0.060	0.402 \pm 0.080	0.182 \pm 0.018 ^e	0.256 \pm 0.030 ^e
Liver	0.822 \pm 0.066	0.727 \pm 0.040	1.041 \pm 0.116	0.611 \pm 0.050
Kidneys	0.350 \pm 0.007	0.045 \pm 0.010	0.611 \pm 0.020 ^e	0.072 \pm 0.010 ^e
Lungs	0.302 \pm 0.004	0.025 \pm 0.000	0.647 \pm 0.119 ^e	0.037 \pm 0.000 ^e
Spleen	0.105 \pm 0.004	0.001 \pm 0.000	0.147 \pm 0.024 ^e	0.001 \pm 0.000
Brain ^c	0.035 \pm 0.004	0.002 \pm 0.000	0.085 \pm 0.028 ^e	0.004 \pm 0.000
Muscle ^c	0.130 \pm 0.059	1.110 \pm 0.390	0.107 \pm 0.039	0.860 \pm 0.310
Fat	0.100 \pm 0.009	-	0.212 \pm 0.049 ^e	-
GI Tract				
plus contents	5.562 \pm 1.926	19.74 \pm 7.350	2.682 \pm 0.539 ^e	5.758 \pm 0.760 ^e
Residual Bile	15.88 \pm 14.72	-	2.920 \pm 0.045 ^e	-
Feces		5.447 \pm 0.560		7.803 \pm 1.200
Urine		68.07 \pm 13.94		52.85 \pm 1.720
Recovery ^d		95.57 \pm 1.517		68.26 \pm 1.105 ^e

^a Mean \pm SE of three (oral) or four (dermal) rabbits.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat, residual bile and skin (including site of application) are not included in the recovery estimates.

^e Significantly different ($p < 0.05$) from oral treatment.

TABLE 11

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO MALE NEW ZEALAND RABBITS^a

Tissue/Excretum	Oral		Dermal	
	Concentration ($\mu\text{g eq/g or ml}$)	Percent of Dose	Concentration ($\mu\text{g eq/g or ml}$)	Percent of Dose
Blood ^b	2.26 (1.82, 2.70)	0.34 (0.30, 0.38)	2.19 (2.40, 1.97)	0.33 (0.34, 0.32)
Liver	8.67 (6.39, 10.95)	0.585 (0.48, 0.69)	7.33 (8.40, 6.26)	0.68 (0.75, 0.61)
Kidneys	3.73 (2.96, 4.50)	0.045 (0.04, 0.05)	6.88 (5.00, 8.75)	0.08 (0.05, 0.11)
Lungs	2.44 (2.12, 2.75)	0.025 (0.02, 0.03)	4.25 (5.60, 2.89)	0.08 (0.04, 0.12)
Spleen	1.21 (1.12, 1.30)	0.002 (0.001, 0.002)	0.96 (1.10, 0.82)	0.001 (0.001, 0.001)
Brain ^c	0.47 (0.13, 0.80)	0.003 (0.001, 0.005)	0.47 (0.60, 0.33)	0.003 (0.003, 0.002)
Muscle ^c	0.66 (0.42, 0.90)	0.565 (0.39, 0.74)	0.62 (0.60, 0.64)	0.54 (0.48, 0.59)
Fat	1.78 (2.26, 1.30)	-	2.76 (1.80, 3.72)	-
GI Tract plus contents	76.78 (31.5, 122.05)	22.66 (11.95, 33.36)	18.99 (14.50, 23.47)	5.83 (4.34, 7.32)
Residual Bile	16.67 (5.34, 28.00)	-	41.03 (26.30, 55.75)	-
Feces		5.08 (6.22, 3.93)		2.75 (2.37, 1.93)
Urine		74.34 (80.44, 68.23)		47.18 (52.03, 42.32)
Recovery ^d		103.63 (99.842, 107.417)		56.86 (60.404, 53.323)

^a Average of two rabbits per treatment. Values from individual animals are shown in parentheses.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat, residual bile and skin (including site of application) are not included in the recovery estimates.

TABLE 12

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ^{14}C -TNT (5 mg/kg) TO MALE BEAGLE DOGS^a

Tissue/Excretum	Oral		Dermal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	0.717 \pm 0.215	1.110 \pm 0.364	0.188 \pm 0.026 ^e	0.263 \pm 0.036 ^e
Liver	3.525 \pm 0.156	2.402 \pm 0.127	0.740 \pm 0.036 ^e	0.503 \pm 0.030 ^e
Kidneys	1.017 \pm 0.183	0.130 \pm 0.012	0.498 \pm 0.031 ^e	0.060 \pm 0.003 ^e
Lungs	0.817 \pm 0.164	0.137 \pm 0.027	0.668 \pm 0.330 ^e	0.128 \pm 0.060
Spleen	0.540 \pm 0.140	0.060 \pm 0.030	0.240 \pm 0.036 ^e	0.016 \pm 0.003 ^e
Brain ^c	0.120 \pm 0.020	0.020 \pm 0.000	0.166 \pm 0.086 ^e	0.030 \pm 0.015
Muscle ^c	0.165 \pm 0.045	1.405 \pm 0.325	0.086 \pm 0.006 ^e	0.683 \pm 0.068 ^e
Fat	0.145 \pm 0.035	-	0.553 \pm 0.263 ^e	-
GI Tract				
plus contents	6.439 \pm 2.266	14.632 \pm 6.498	1.293 \pm 0.138 ^e	1.682 \pm 0.130 ^e
Residual Bile	59.825 \pm 6.325	-	39.996 \pm 3.328 ^e	-
Feces		8.995 \pm 0.025		1.710 \pm 0.380 ^e
Urine		70.50 \pm 2.955		11.730 \pm 1.648 ^e
Recovery ^d		99.391 \pm 6.032		16.807 \pm 1.244 ^e

^a Mean \pm SE of three dogs per treatment.

^b Based on 7% body weight.

^c Based on 40% of body weight.

^d Fat, residual bile and skin (including site of application) are not included in the recovery estimates.

^e Significantly different ($p < 0.05$) from oral treatment.

TABLE 13

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 HR AFTER ORAL OR DERMAL
ADMINISTRATION OF ¹⁴C-TNT (50 mg/kg) TO MALE BEAGLE DOGS^a

Tissue/Excretum	Oral		Dermal	
	Concentration (μ g eq/g or ml)	Percent of Dose	Concentration (μ g eq/g or ml)	Percent of Dose
Blood ^b	29.225	5.410	3.095	0.435
Liver	22.600	1.503	12.165	0.680
Kidneys	9.850	0.132	3.630	0.040
Lungs	8.665	0.145	2.360	0.035
Spleen	19.820	0.298	2.800	0.029
Brain ^c	2.180	0.056	0.542	0.009
Muscle ^c	1.606	1.690	0.552	0.441
Fat	5.230	-	6.025	-
GI Tract plus contents	14.392	1.667	16.065	1.690
Residual Bile	3,129.000	-	545.460	-
Feces		22.235		0.770
Urine		61.030		11.806
Recovery ^d		94.166		15.935

^a One dog per treatment.

^b Based on 7% of body weight.

^c Based on 40% body weight.

^d Fat, residual bile and skin (including site of application)
are not included in the recovery estimates.

TABLE 14

BILE/LIVER, LIVER/BLOOD, AND BILE/BLOOD CONCENTRATION RATIOS 24 HR AFTER
ORAL OR DERMAL ADMINISTRATION OF ^{14}C -TNT TO MALE RABBITS AND DOGS^a

Species	Route	Dose (mg/kg)	Concentration (μg eg/g or ml)			Ratio	
			Bile	Liver	Blood	Bile/Liver	Bile/Blood
Rabbit	Oral	5	16	0.82	0.26	19	3.2
		50	17	8.71	1.90	2	4.6
	Dermal	5	3	1.04	0.18	3	5.8
		50	41	7.20	1.10	6	6.5
Dog	Oral	5	60	3.53	0.72	17	4.9
		50	3,129	22.60	29.23	139	0.8
	Dermal	5	40	0.74	0.19	54	3.9
		50	545	12.17	3.10	45	3.9
							61
							9
							16
							37
							83
							107
							211
							176

^a The ratios were calculated from liver concentrations which were not corrected for biliary ^{14}C content.

TABLE 15

TISSUE-TO-BLOOD CONCENTRATION RATIOS IN MALE RATS, MICE, RABBITS, AND DOGS
AT 24 HR FOLLOWING ORAL OR DERMAL TREATMENT WITH ^{14}C -TNT

Tissue	Rats		Mice		Rabbits		Dogs	
	Oral	Dermal	Oral	Dermal	Oral	Dermal	Oral	Dermal
Liver	4.2	2.0	5.2	2.7	3.2	5.8	4.9	3.9
Kidneys	3.3	2.1	3.3	2.5	1.3	3.4	1.4	2.6
Lungs	1.2	1.0	1.7	1.1	1.2	3.6	1.1	3.5
Spleen	0.6	0.4	1.2	0.5	0.4	0.8	0.8	1.3
Brain	0.3	0.6	0.5	0.5	0.1	0.5	0.2	0.9
Muscle	0.5	0.4	0.5	0.6	0.5	0.6	0.2	0.5
Fat	0.6	1.7	0.8	2.6	0.4	1.2	0.2	2.9
Blood ($\mu\text{g/ml}$)	1.0 (1.77)	1.0 (1.44)	1.0 (0.93)	1.0 (1.23)	1.0 (0.26)	1.0 (0.18)	1.0 (0.72)	1.0 (0.18)

TABLE 16

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 4 HR AFTER ORAL OR INTRATRACHEAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO MALE SPRAGUE-DAWLEY RATS^a

Tissue/Excretum	Oral		Intratracheal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	9.59 \pm 1.07	1.34 \pm 0.14	15.62 \pm 1.21 ^e	2.24 \pm 0.11 ^e
Liver	12.21 \pm 1.48	0.98 \pm 0.10	13.50 \pm 0.48	1.13 \pm 0.05
Kidneys	11.74 \pm 1.01	0.23 \pm 0.01	17.48 \pm 1.34 ^e	0.37 \pm 0.05 ^e
Lungs	44.00 \pm 7.86	0.38 \pm 0.07	35.70 \pm 4.18	0.28 \pm 0.03
Spleen	3.35 \pm 1.31	0.01 \pm 0.00	3.19 \pm 0.16	0.01 \pm 0.00
Brain ^c	4.35 \pm 0.73	0.05 \pm 0.01	6.48 \pm 0.59 ^e	0.08 \pm 0.00 ^e
Muscle ^c	2.44 \pm 0.62	1.95 \pm 0.49	4.92 \pm 0.46 ^e	3.93 \pm 0.37 ^e
Fat	30.80 \pm 2.40	-	82.41 \pm 5.64 ^e	-
GI Tract, No Bile Collected	499.0 \pm 41.35	73.70 \pm 6.82	81.65 \pm 7.21 ^e	18.24 \pm 1.03 ^e
GI Tract, (Bile Collected)	(412.0 \pm 37.82)	(68.29 \pm 3.70)	(12.75 \pm 1.02) ^e	(1.79 \pm 0.02) ^e
Urine, No Bile Collected		14.63 \pm 2.16		19.32 \pm 3.21
Urine (Bile Collected)		(10.73 \pm 1.52)		(17.50 \pm 0.90) ^e
Bile		11.57 \pm 2.61		19.75 \pm 1.43 ^e
Recovery, No Bile Collected ^d		93.27 \pm 5.01		45.60 \pm 3.78 ^e
Recovery (Bile Collected) ^d		(95.53 \pm 3.22)		(47.06 \pm 1.31) ^e

a Mean \pm SE of five (oral) or six (intratracheal) rats. Three (oral) or four (intratracheal) rats had cannulated bile ducts.

b Based on 7% of body weight.

c Based on 40% of body weight.

d Fat is not included in the recovery estimates.

e Significantly different ($p < 0.05$) from oral treatment.

TABLE 17

TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 4 HR AFTER ORAL OR INTRATRACHEAL
ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO FEMALE SPRAGUE-DAWLEY RATS^a

Tissue/Excretum	Oral		Intratracheal	
	Concentration (μg eq/g or ml)	Percent of Dose	Concentration (μg eq/g or ml)	Percent of Dose
Blood ^b	18.33 \pm 2.26	2.78 \pm 0.30	30.69 \pm 2.14 ^e	4.29 \pm 0.32 ^e
Liver	9.61 \pm 1.03	0.75 \pm 0.11	14.25 \pm 0.78	1.13 \pm 0.09
Kidneys	19.14 \pm 2.41	0.36 \pm 0.05	23.15 \pm 1.67	0.46 \pm 0.03
Lungs	21.35 \pm 2.70	0.25 \pm 0.04	23.58 \pm 3.89 ^e	0.27 \pm 0.03
Spleen	2.03 \pm 0.62	0.01 \pm 0.33	5.84 \pm 0.48 ^e	0.03 \pm 0.00 ^e
Brain ^c	9.44 \pm 0.79	0.13 \pm 0.02	16.21 \pm 1.21 ^e	0.27 \pm 0.03 ^e
Muscle ^c	6.95 \pm 0.82	4.62 \pm 0.53	11.34 \pm 0.79 ^e	8.42 \pm 0.61 ^e
Fat	96.31 \pm 8.21	-	154.74 \pm 13.68 ^e	-
GI Tract, No Bile Collected	527.3 \pm 37.2	79.02 \pm 5.23	39.94 \pm 4.22 ^e	12.06 \pm 1.13 ^e
GI Tract (Bile Collected)	(420.0 \pm 46.2)	(64.22 \pm 7.21)	(16.63 \pm 1.12) ^e	(2.92 \pm 0.27) ^e
Urine, No Bile Collected		10.01 \pm 1.47		13.23 \pm 2.01
Urine (Bile Collected)		(8.42 \pm 1.13)		(12.68 \pm 0.83) ^e
Bile		9.67 \pm 0.74		14.51 \pm 1.20 ^e
Recovery, No Bile Collected ^d		97.93 \pm 6.21		40.16 \pm 2.03 ^e
Recovery (Bile Collected)		(91.21 \pm 6.37)		(44.98 \pm 2.11) ^e

^a Mean \pm S.E. of five (oral) or six (intratracheal) rats. Three (oral) or four (intratracheal) rats had cannulated bile ducts.

^b Based on 7% of body weight.

^c Based on 40% of body weight.

^d Fat is not included in the recovery estimates.

^e Significantly different ($p < 0.05$) from oral treatment.

TABLE 18

BILE/LIVER, LIVER/BLOOD, AND BILE/BLOOD CONCENTRATION RATIOS 24 HR AFTER ORAL
OR INTRATRACHEAL ADMINISTRATION OF ^{14}C -TNT (50 mg/kg) TO MALE RATS^a

Route	Time After Dosing (hr)	Concentration ($\mu\text{g eg/g}$ or ml)			Ratio	
		Bile	Liver	Blood	Bile/Liver	Bile/Blood
Oral	0.25	345		7.2		48
	0.5	567		7.5		76
	1.0	547		9.0		61
	2.0	484		9.7		50
	4.0	413	12.2	9.6	34	43
Intratracheal	0.25	1,687		35.9		47
	0.5	1,903		26.6		72
	1.0	1,460		23.6		62
	2.0	972		19.4		50
	4.0	689	13.5	15.7	51	44
						0.86

^a The ratios were calculated from liver concentrations which were not corrected for biliary ^{14}C content.

TABLE 19

TISSUE-TO-BLOOD CONCENTRATION RATIOS IN RATS AT 4 HR FOLLOWING
ORAL OR INTRATRACHEAL ADMINISTRATION OF ^{14}C -TNT

<u>Tissue</u>	<u>Males</u>		<u>Females</u>	
	<u>Oral</u>	<u>Intratracheal</u>	<u>Oral</u>	<u>Intratracheal</u>
Liver	1.3	0.9	0.5	0.5
Kidneys	1.2	1.1	1.0	0.8
Lungs	4.6	2.3	1.2	0.8
Spleen	0.3	0.2	0.1	0.2
Brain	0.5	0.4	0.5	0.5
Muscle	0.3	0.3	0.4	0.4
Fat	3.2	5.3	5.3	5.0
Blood ($\mu\text{g/ml}$)	1.0 (9.59)	1.0 (15.62)	1.0 (18.33)	1.0 (30.69)

TABLE 20

ETHYL ACETATE EXTRACTABLE RADIOACTIVITY FROM URINE
INCUBATED WITHOUT OR WITH β -GLUCURONIDASE

Source of Urine	Route of Administration	Percent of Total Radioactivity		Ratio B/A
		(A) Without β -Glucuronidase	(B) With β -Glucuronidase	
Male rats	Oral	19.3	56.2	2.91
	Dermal	22.6	52.7	2.33
	Oral ^a	46.1	57.6	1.25
	Intratracheal ^a	52.3	66.9	1.28
Female rats	Oral	23.4	58.7	2.51
	Dermal	29.2	53.4	1.83
	Oral ^a	43.6	60.2	1.38
	Intratracheal ^a	38.7	64.6	1.67
Male mice	Oral	45.3	59.8	1.32
	Dermal	47.1	57.0	1.21
Male rabbit	Oral	29.0	55.1	1.90
	Dermal	36.3	55.2	1.52
Male dog	Oral	23.4	54.3	2.32
	Dermal	29.0	52.5	1.81

^a Urine collected from bile duct-cannulated rats.

TABLE 21
ETHYL ACETATE EXTRACTABLE RADIOACTIVITY FROM BILE
INCUBATED WITHOUT OR WITH β -GLUCURONIDASE

Source of Bile	Route of Administration	Percent of Total Radioactivity		Ratio B/A
		(A) Without β -Glucuronidase	(B) With β -Glucuronidase	
Male Rat	Oral ^a	9.6	39.7	4.14
	Intratracheal ^a	12.2	45.3	3.71
Male Rabbit	Oral ^b	16.3	36.8	2.62
	Dermal ^b	14.1	40.9	2.90
Male Dog	Oral ^b	19.2	65.7	3.42
	Dermal ^b	22.8	71.4	3.13

^a Collected from bile duct-cannulated rats.

^b Residual bile.

TABLE 22

RESOLUTION OF TNT AND SOME POTENTIAL METABOLITES
BY THIN-LAYER CHROMATOGRAPHY

Compound	Solvent System and R _f Values ^a				
	I	II	V	VII	IX
1. Trinitrotoluene (TNT)	0.706	0.524	0.612	0.742	0.638
2. Trinitrobenzyl alcohol	0.699	0.315	0.455	0.521	0.405
3. Trinitrobenzoic acid	0.436	0.018	0.006	0.077	0.050
4. 4-Amino-2,6-dinitrotoluene	0.661	0.339	0.376	0.497	0.380
5. 2-Amino-4,6-dinitrotoluene	0.667	0.321	0.303	0.485	0.374
6. 4,6-Diamino-2-nitrotoluene	0.536	0.089	0.112	0.166	0.123
7. 2,6-Diamino-4-nitrotoluene	0.528	0.074	0.095	0.110	0.074
8. 4-Hydroxylamino-2,6-dinitrotoluene	0.712	0.213	0.260	0.368	0.294
9. 2-Hydroxylamino-4,6-dinitrotoluene	0.687	0.343	0.308	0.490	0.393
10. 2,6,2,6-Tetranitro-4,4-azoxytoluene	0.760	0.645	0.650	0.791	0.650

^a Solvent systems are:

- (I) n-Butanol:acetic acid:water (10:1:1, v/v)
- (II) Benzene:acetic acid (9:1, v/v)
- (V) Benzene:ethylacetate (4:1, v/v)
- (VII) Benzene:acetic acid (4:1, v/v)
- (IX) Toluene:acetic acid (4:1, v/v)

TABLE 23

RESOLUTION OF TNT AND SOME POTENTIAL METABOLITES BY GAS CHROMATOGRAPHY

Compound	Retention Time (min)	
	Column A ^a	Column B ^b
TNT	6.88	1.56
Trinitrobenzyl alcohol	6.3	1.6
4-Amino-2,6-dinitrotoluene	13.4	5.9
2-Amino-4,6-dinitrotoluene	16.2	7.5
4,6-Diamino-2-nitrotoluene	12.5	4.7
2,6-Diamino-4-nitrotoluene	16.3	6.6

^a 10% VC-W982 on 80-100 mesh WAW-DMCS.

^b 1.5% DC-LSX 30295 + 1.5% XE60 on 60-80 mesh gas chromatograph Q.

TABLE 24

RESOLUTION OF TNT AND SOME POTENTIAL METABOLITES
BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY^a

Compound	Retention Time in Minutes (Relative to TNT)			
	System 1	System 2	System 3	System 4
Trinitrotoluene (TNT)	10.3 (1.0)	16.7 (1.0)	26.8 (1.0)	45.3 (1.0)
Trinitrobenzyl alcohol	17.2 (1.67)	15.5 (0.93)	11.0 (0.41)	17.0 (0.38)
2-Amino-4,6-dinitrotoluene	27.8 (2.70)	16.1 (0.96)	56.1 (2.09)	72.2 (1.59)
4-Amino-2,6-dinitrotoluene	29.9 (2.90)	16.3 (0.98)	54.4 (2.03)	71.1 (1.57)
2,6-Diamino-4-nitrotoluene	33.9 (3.29)	16.2 (0.97)	8.7 (0.33)	13.5 (0.3)
4,6-Diamino-2-nitrotoluene	40.7 (3.95)	14.0 (0.84)	11.0 (0.41)	17.0 (0.38)
2-Hydroxylamino-4,6-dinitrotoluene		16.4 (0.98)	54.4 (2.03)	71.1 (1.57)
4-Hydroxylamino-2,6-dinitrotoluene		16.8 (1.0)	60.3 (2.25)	81.2 (1.79)

^a For a description of the systems used see text.

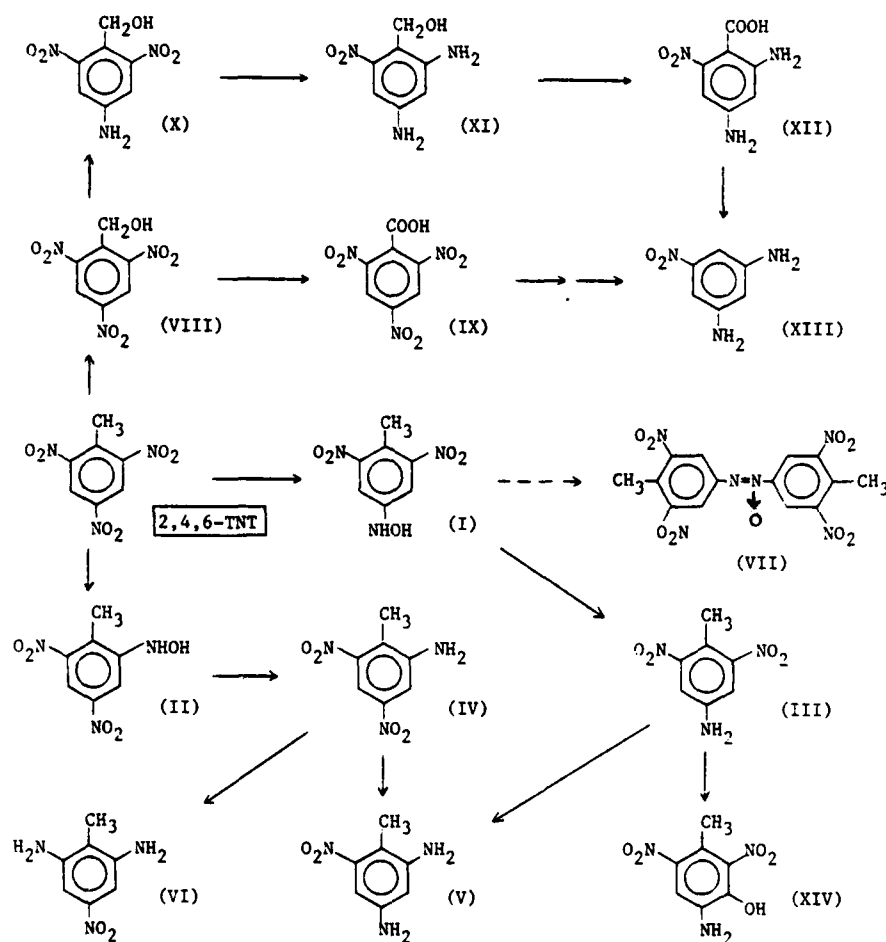


Figure 1: Schematic Presentation for Some Possible Biotransformation Products of 2,4,6-TNT

- | | |
|--|---|
| (I) 4-Hydroxylamino-2,6-dinitrotoluene | (VIII) 2,4,6-Trinitrobenzylalcohol |
| (II) 2-Hydroxylamino-4,6-dinitrotoluene | (IX) Trinitrobenzoic acid |
| (III) 4-Amino-2,6-dinitrotoluene | (X) 4-Amino-2,6-dinitrobenzylalcohol |
| (IV) 2-Amino-4,6-dinitrotoluene | (XI) 2,4-Diamino-6-nitrobenzylalcohol |
| (V) 4,6-Diamino-2-nitrotoluene | (XII) 2,4-Diamino-6-nitrobenzoic acid |
| (VI) 2,6-Diamino-4-nitrotoluene | (XIII) 5-Nitro- <u>m</u> -phenylenediamine |
| (VII) 2,6,2',6'-Tetranitro-4,4'-azoxytoluene | (XIV) 4-Amino-2,6-dinitro- <u>m</u> -cresol |

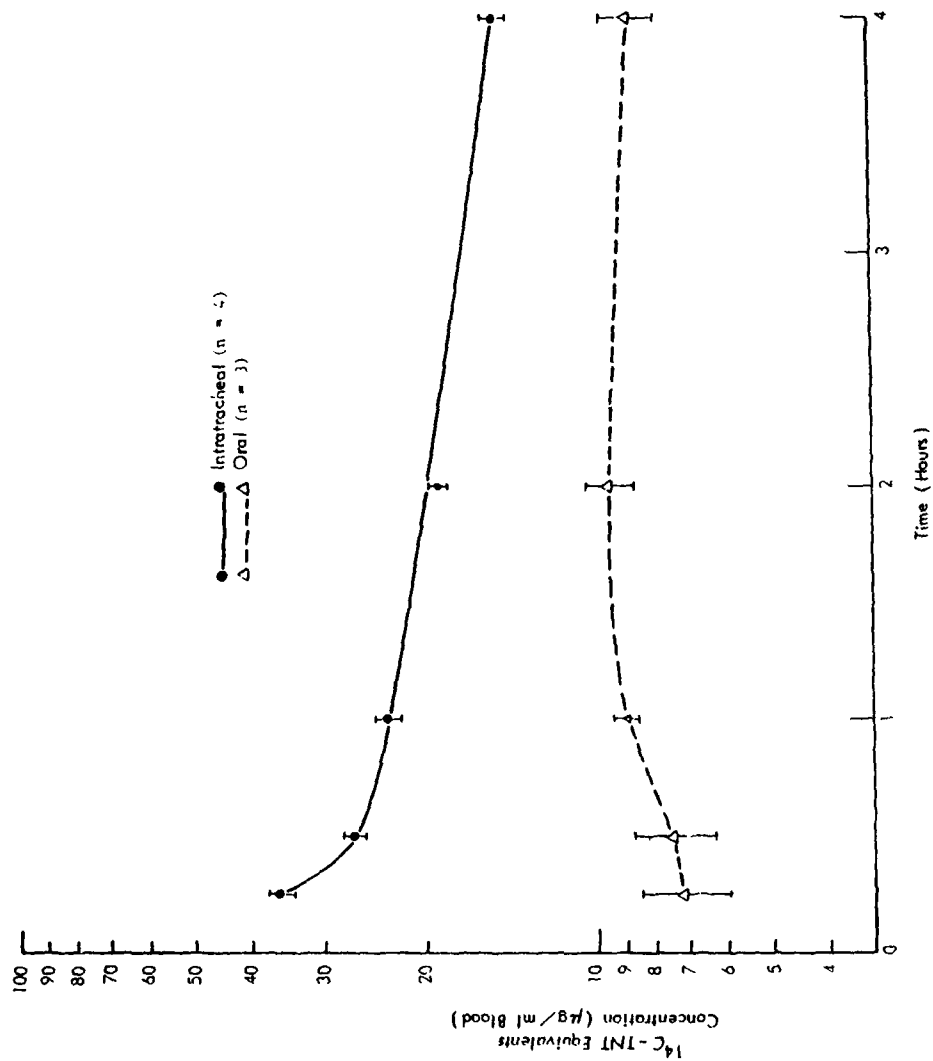


Figure 2: Levels of Radioactivity in Blood Following Oral or Intratracheal Administration of ^{14}C -TNT (50 mg/kg) to Male Sprague-Dawley Rats. Each point is the mean \pm SE of 3 to 4 rats.

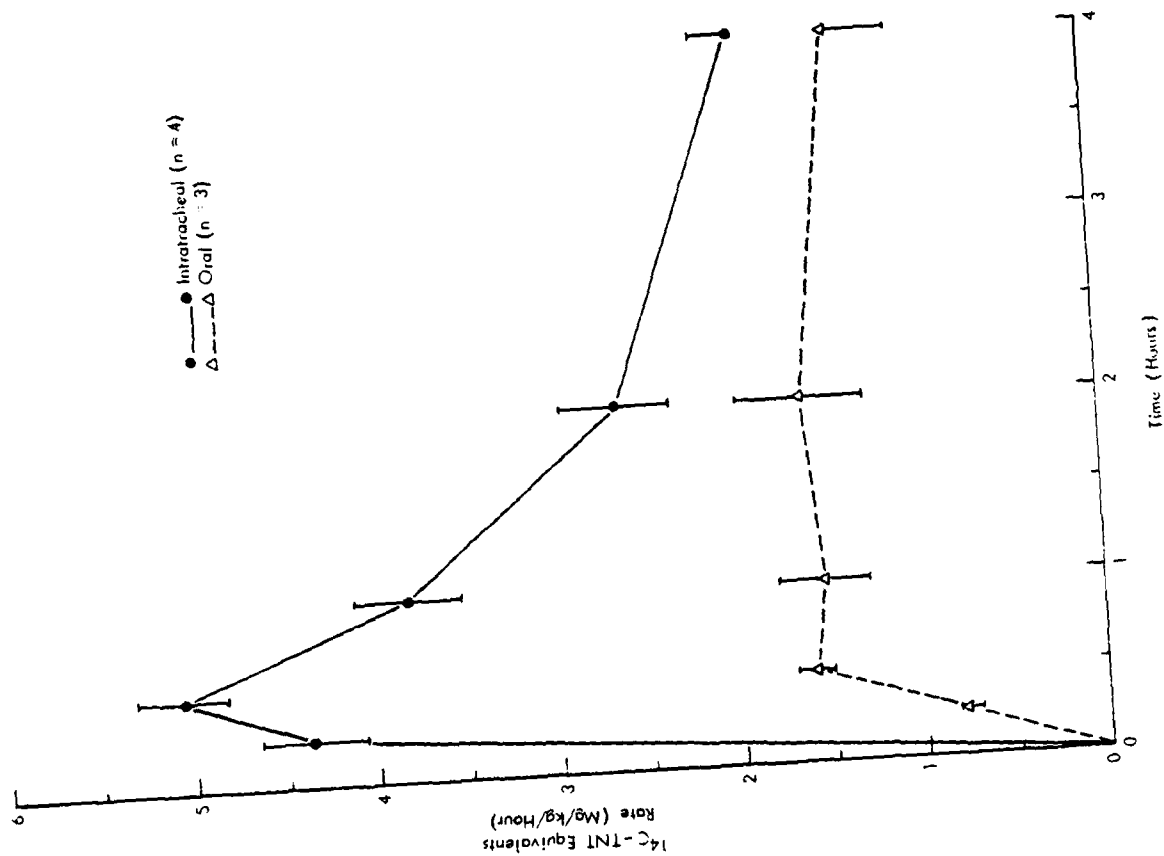


Figure 3: Rates of Excretion of Radioactivity in Bile Following Oral or Intratracheal Administration of ^{14}C -TNT (50 mg/kg) to Male Sprague-Dawley Rats. Each point is the mean \pm SE of 3 to 4 rats.

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SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM OF 2,4,6-ETC(U)

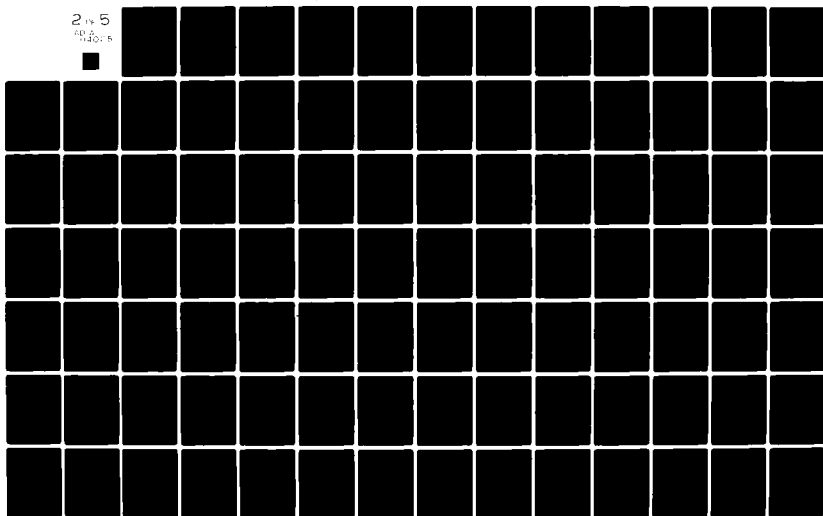
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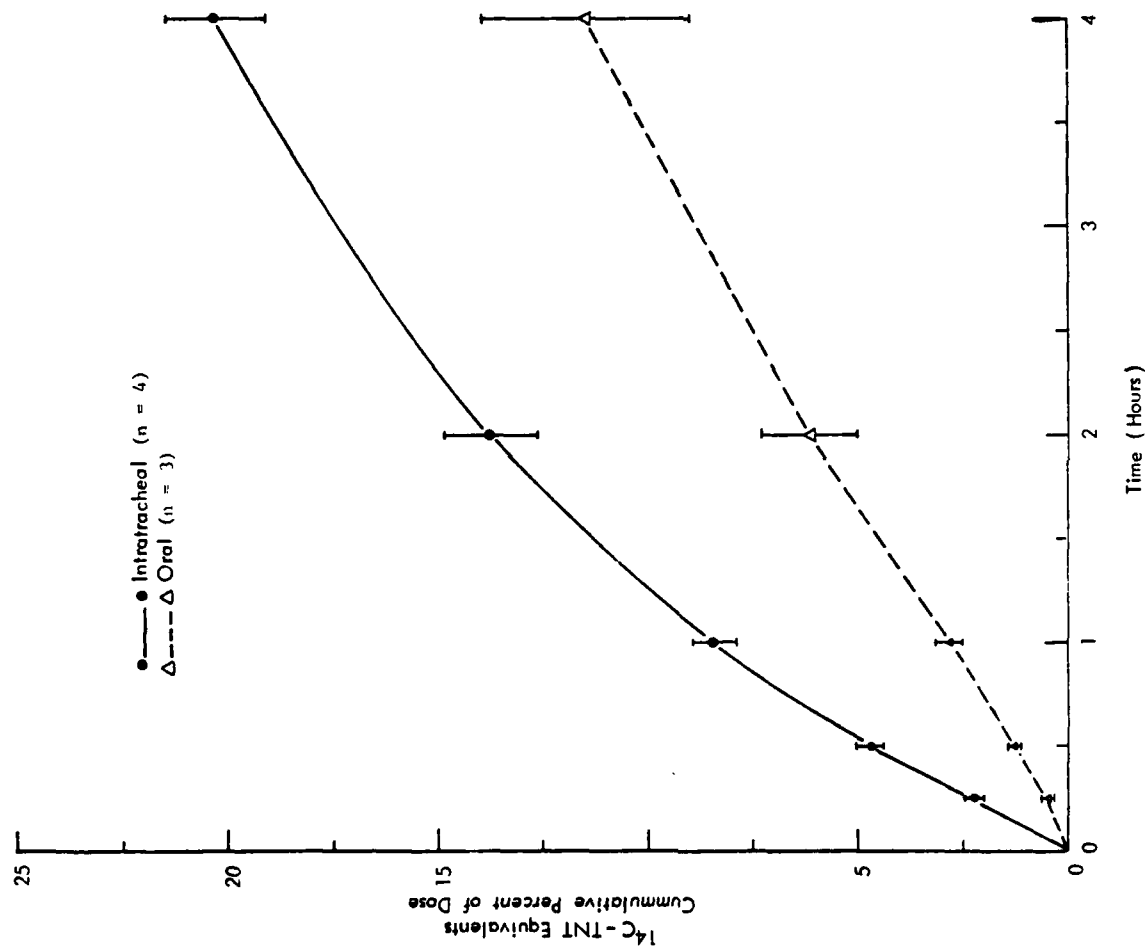


Figure 4: Cumulative Excretion of Radioactivity in Bile Following Oral or Intratracheal Administration of ^{14}C -TNT (50 mg/kg) to Male Sprague-Dawley Rats. Each point is the mean \pm SE of 3 to 4 rats.

MIXTURE OF TNT AND POTENTIAL METABOLITES

Adjust of pH 7, Extract with Ether

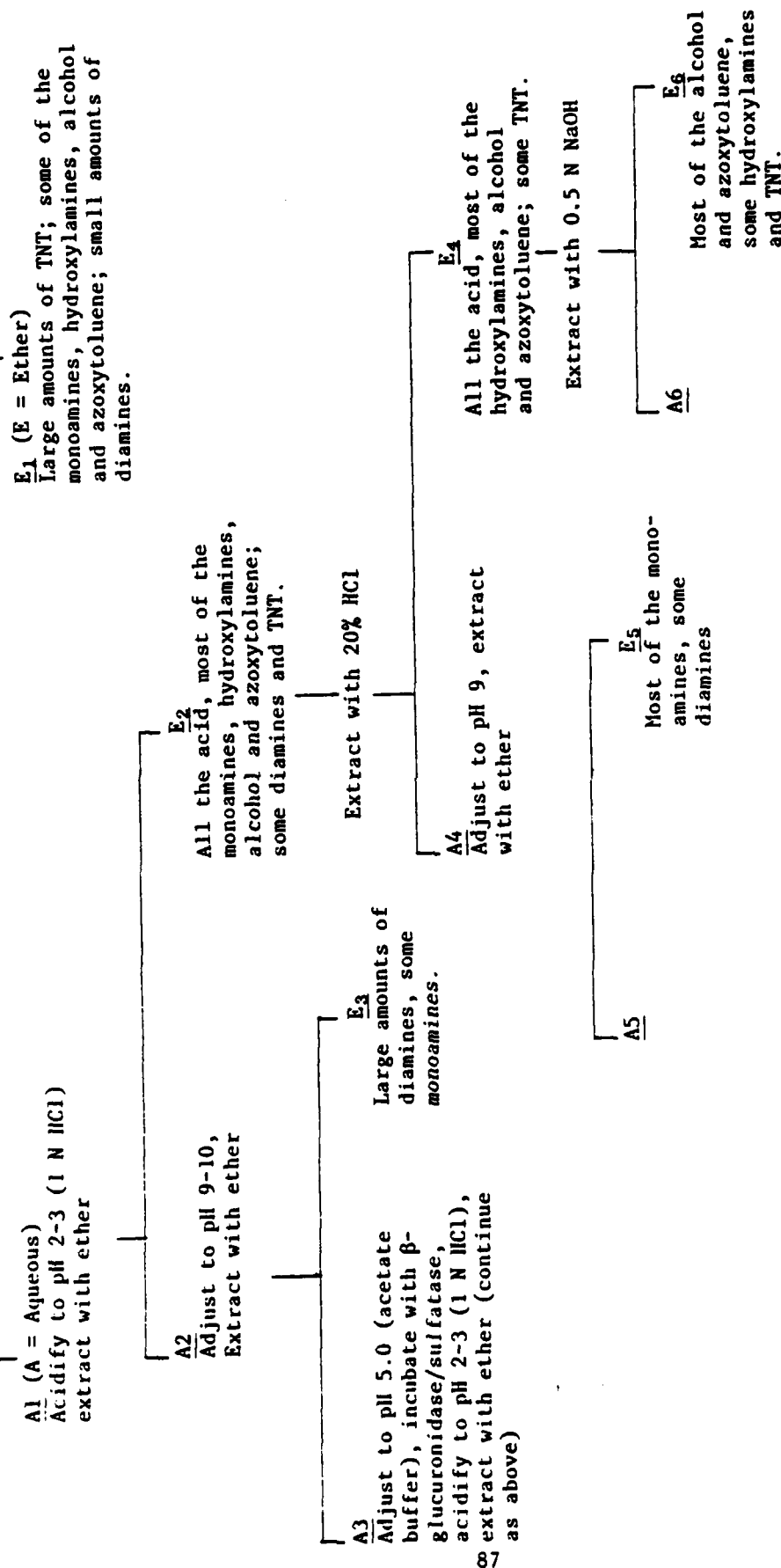


Figure 5-a: Fractionation of a Mixture of TNT and Nine Potential Metabolites by Extraction with Ether at Different pH conditions. The mixture consisted of the following:

- | | |
|-------------------------------|--|
| 1. TNT | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

24 HR URINE

Rat, Mouse, Rabbit, Dog

Adjust to pH 7.0, extract with ether (3 x 100 ml)

A₁ (A = Aqueous)

Rats, oral 95.4, dermal 89.3
Mice, oral 91.4, dermal 91.5
Rabbits, oral 94.2, dermal 93.3
Dogs, oral 88.9, dermal 83.9

E₁ (E = Ether)

Rats, oral 4.6, dermal 10.7
Mice, oral 8.6, dermal 8.5
Rabbits, oral 5.8, dermal 6.7
Dogs, oral 11.0, dermal 16.1

Adjust to pH 2-3 (1N HCl), extract with ether (3 x 100 ml)

A₂

Rats, oral 47.9, dermal 53.2
Mice, oral 40.1, dermal 53.1
Rabbits, oral 61.8, dermal 54.8
Dogs, oral 50.3, dermal 61.1

E₂

Rats, oral 47.5, dermal 36.1
Mice, oral 51.4, dermal 38.5
Rabbits, oral 32.6, dermal 38.5
Dogs, oral 38.6, dermal 22.8

Adjust to pH 9-10 (1N NaOH),
extract with ether (3 x 100 ml)

Extract with 20% HCl (2 x 50 ml)

A₃

Rats, oral 46.5, dermal 52.6
Mice, oral 37.9, dermal 51.7
Rabbits, oral 59.1, dermal 53.0
Dogs, oral 47.7, dermal 59.2
Adjust to pH 5.0 (acetate buffer),
incubate with β -glucuronidase,
acidify to pH 2-3 (in HCl),
extract with ether (continue
as above)

E₃

Rats, oral 1.4, dermal 0.6
Mice, oral 2.3, dermal 1.3
Rabbits, oral 2.7, dermal 1.8
Dogs, oral 2.6, dermal 1.9

A₄

Rats, oral 9.7, dermal 11.3
Mice, oral 14.3, dermal 11.8
Rabbits, oral 7.3, dermal 11.0
Dogs, oral 11.2, dermal 5.1

E₄

Rats, oral 37.8, dermal 24.8
Mice, oral 37.0, dermal 26.7
Rabbits, oral 24.6, dermal 27.5
Dogs, oral 27.5, dermal 17.7

Adjust to pH 9 (5N NaOH),
extract with ether (2 x 50 ml)

Adjust to pH 9 (5N NaOH),
extract with ether (2 x 50 ml)

Extract with 0.5N NaOH (2 x 50 ml)

A₅

Rats, oral 7.9, dermal 10.3
Mice, oral 8.1, dermal 8.4
Rabbits, oral 4.7, dermal 7.7
Dogs, oral 6.6, dermal 3.9

E₅

Rats, oral 1.8, dermal 1.0
Mice, oral 6.2, dermal 3.4
Rabbits, oral 2.6, dermal 3.2
Dogs, oral 4.6, dermal 1.3

A₆

Rats, oral 31.8, dermal 22.6
Mice, oral 26.8, dermal 18.7
Rabbits, oral 16.7, dermal 16.3
Dogs, oral 20.8, dermal 13.3

E₆

Rats, oral 6.0, dermal 2.2
Mice, oral 10.2, dermal 8.0
Rabbits, oral 15.1, dermal 11.3
Dogs, oral 6.7, dermal 4.4

Figure 5-b: Fractionation of 24 Hr Urine Obtained from Animals Treated Orally or Dermal with ¹⁴C-TNT
(Values indicate the percentage of extractable radioactivity in each fraction.)

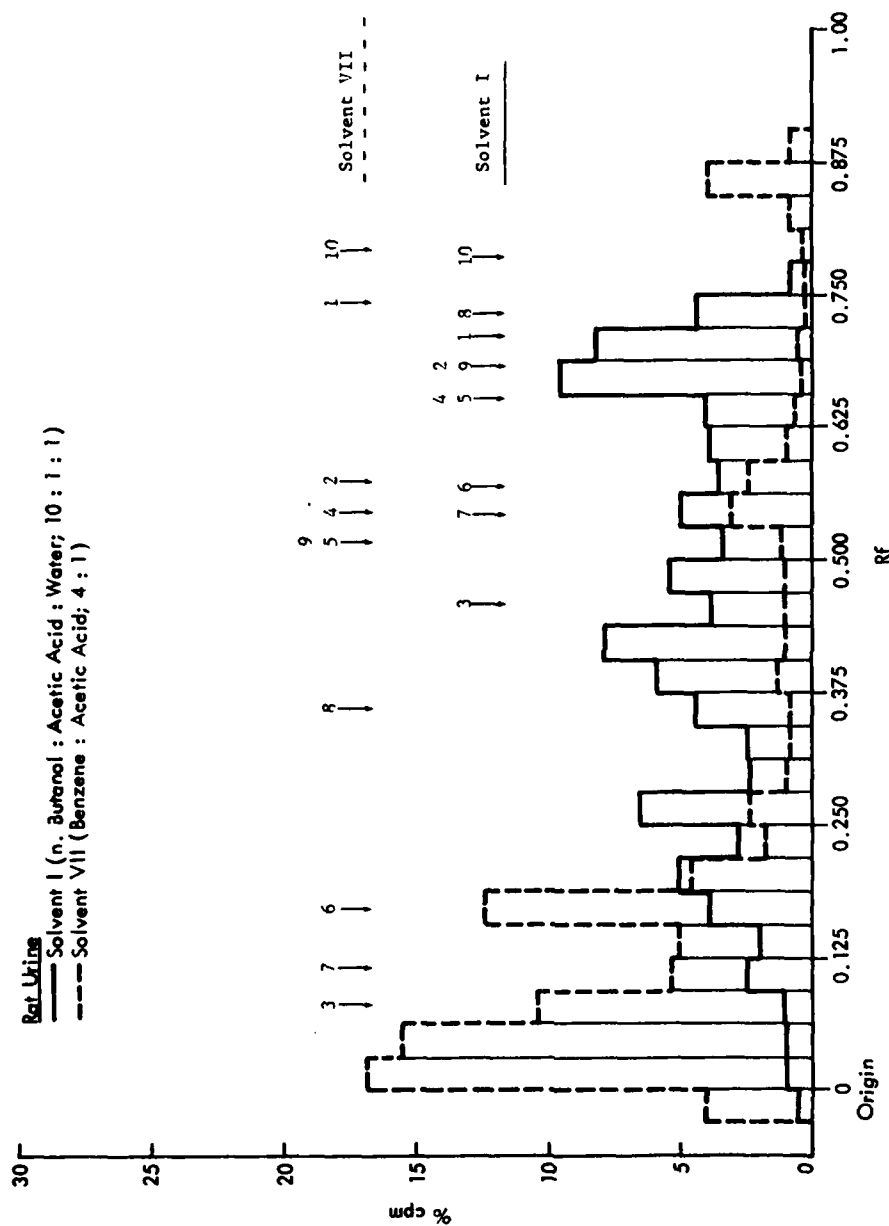


Figure 6: TLC of the Ethyl Acetate Extractable Products Obtained from Urine of Rats Treated Orally with 14C-TNT (100 mg/kg).

TNT and potential metabolites available as references are:

1. TNT
2. Trinitrobenzyl alcohol
3. Trinitrobenzoic acid
4. 4-Amino-2,6-dinitrotoluene
5. 2-Amino-4,6-dinitrotoluene
6. 4,6-Diamino-2-nitrotoluene
7. 2,6-Diamino-4-nitrotoluene
8. 4-Hydroxylamino-2,6-dinitrotoluene
9. 2-Hydroxylamino-4,6-dinitrotoluene
10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene

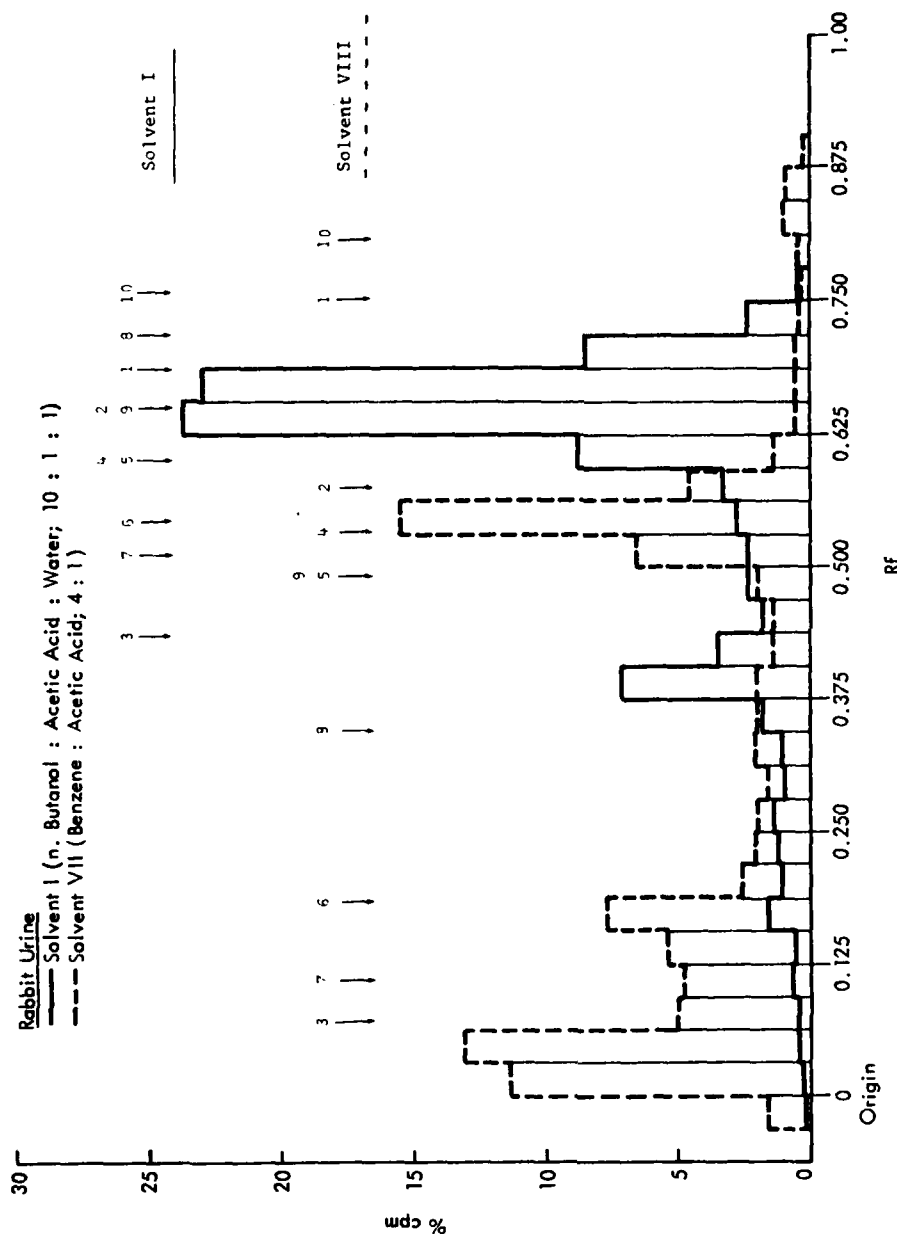


Figure 7: TLC of the Ethyl Acetate Extractable Products Obtained from

Urine of Rabbits Treated Orally with ^{14}C -TNT (5 mg/kg).

TNT and potential metabolites available as references are:

1. TNT
2. Trinitrobenzyl alcohol
3. Trinitrobenzoic acid
4. 4-Amino-2,6-dinitrotoluene
5. 2-Amino-4,6-dinitrotoluene
6. 4,6-Diamino-2-nitrotoluene
7. 2,6-Diamino-4-nitrotoluene
8. 4-Hydroxylamino-2,6-dinitrotoluene
9. 2-Hydroxylamino-4,6-dinitrotoluene
10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene

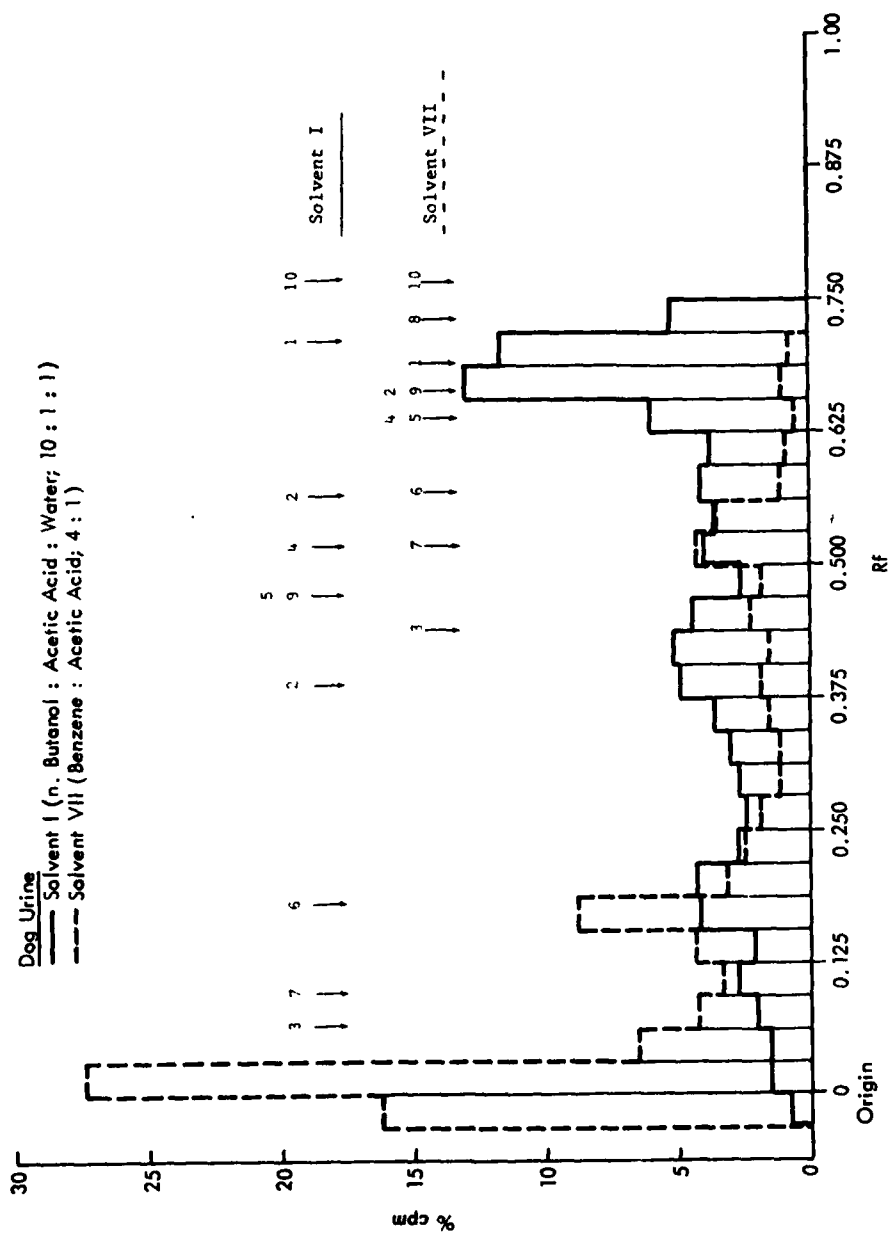


Figure 8: TLC of the Ethyl Acetate Extractable Products Obtained from Urine of Dogs Treated Orally With ^{14}C -TNT (5 mg/kg).

TNT and potential metabolites available as references are:

1. TNT
2. Trinitrobenzyl alcohol
3. Trinitrobenzoic acid
4. 4-Amino-2,6-dinitrotoluene
5. 2-Amino-4,6-dinitrotoluene
6. 4,6-Diamino-2-nitrotoluene
7. 2,6-Diamino-4-nitrotoluene
8. 4-Hydroxylamino-2,6-dinitrotoluene
9. 2-Hydroxylamino-4,6-dinitrotoluene
10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene

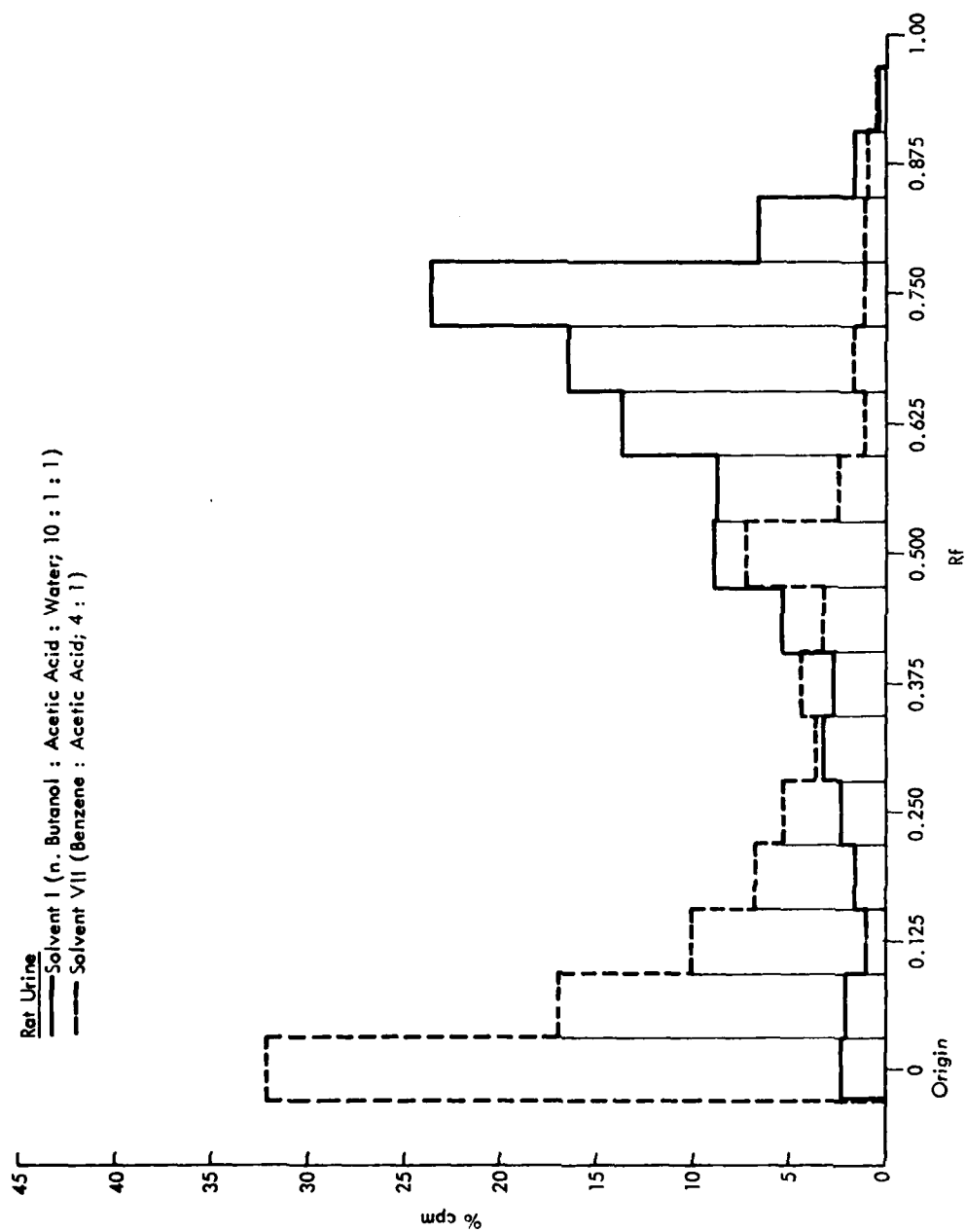


Figure 9-a: TLC of Rat Urine Obtained after Oral Administration of ^{14}C -TNT (100 mg/kg). Urine was incubated with acetate buffer (pH 5.0) for 24 hr then extracted with ethyl acetate.

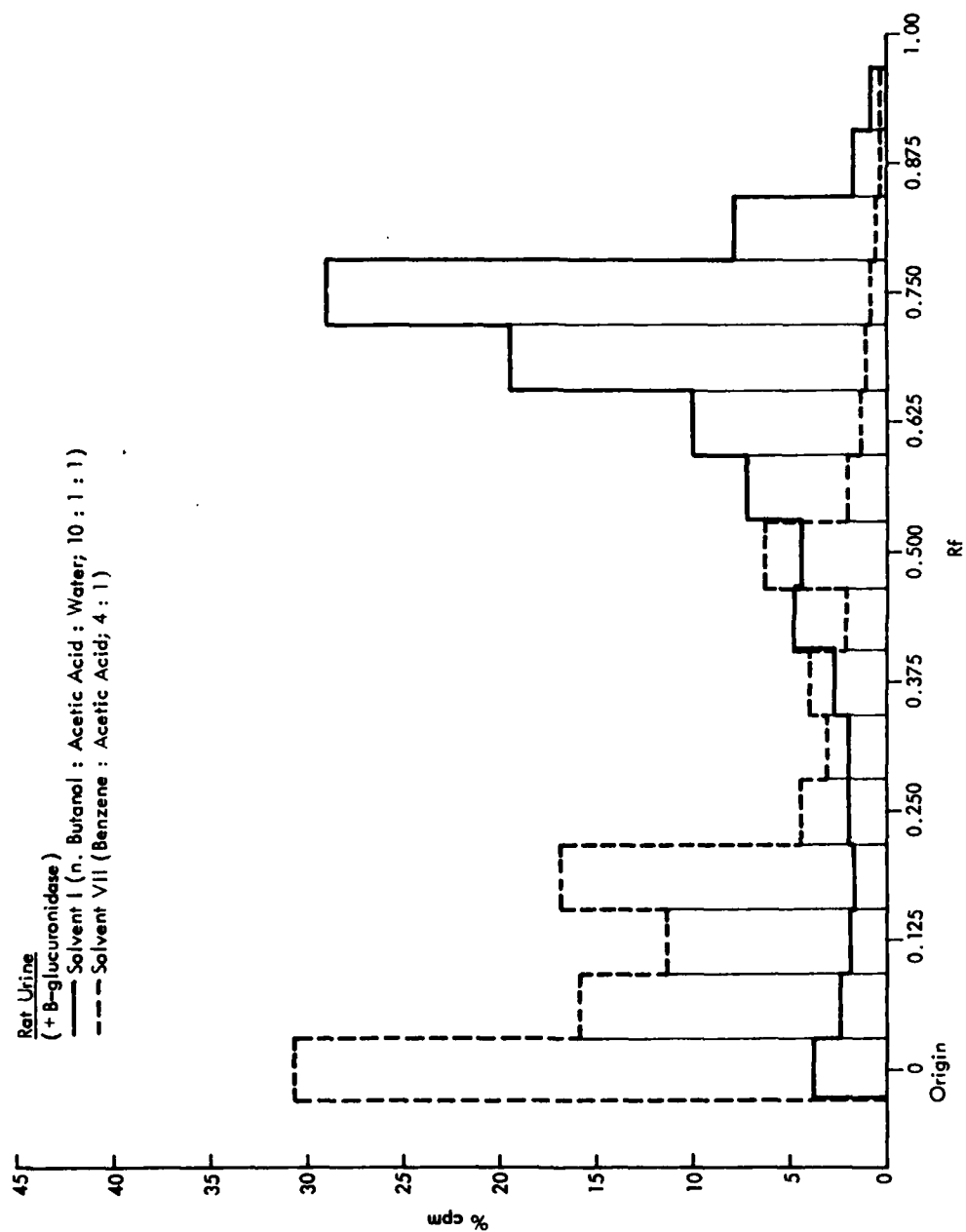


Figure 9-b: TLC of Rat Urine Obtained after Oral Administration of ^{14}C -TNT (100 mg/kg). Urine was incubated with acetate buffer (pH 5.0) and β -glucuronidase for 24 hr then extracted with ethyl acetate.

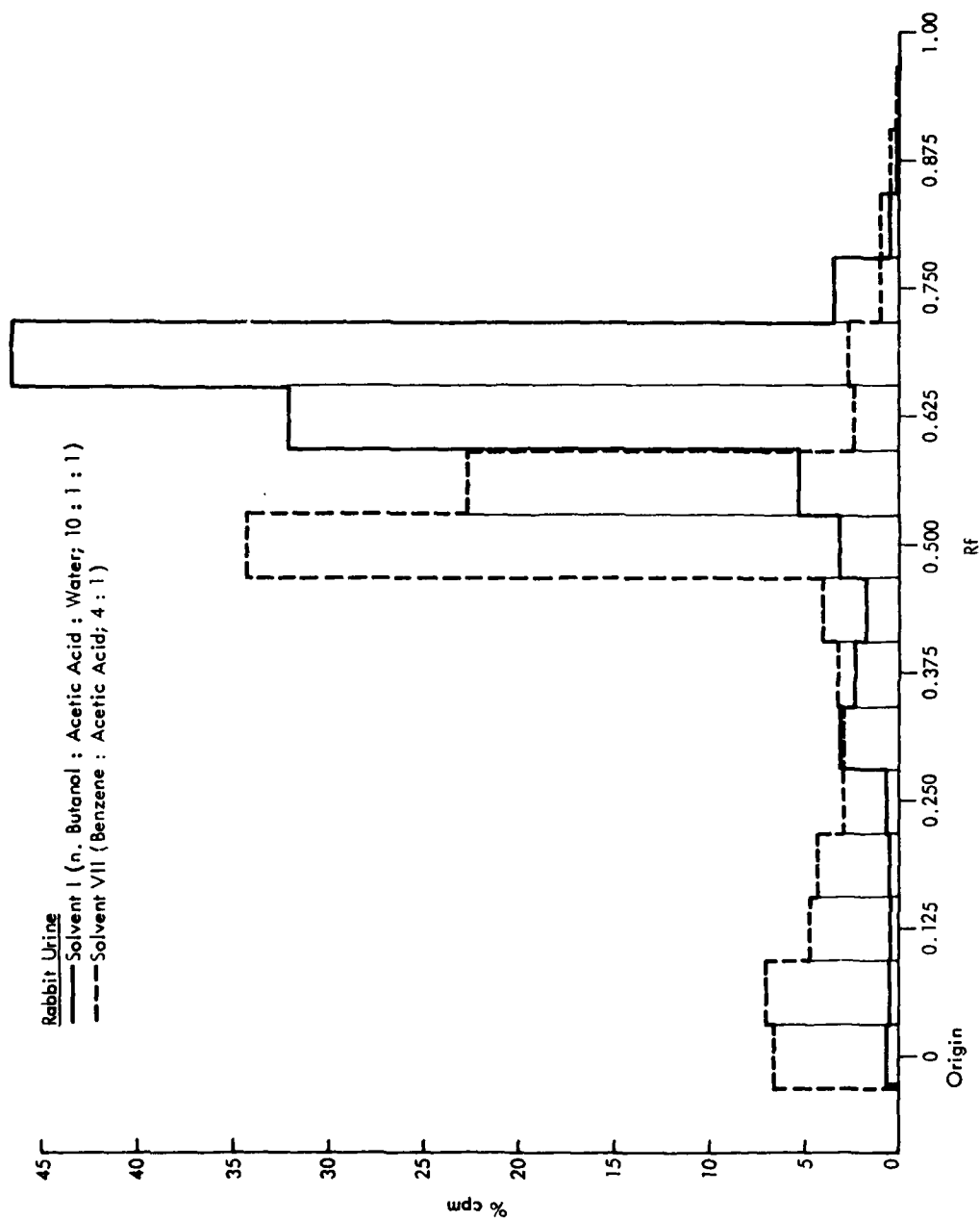


Figure 10-a: TLC of Rabbit Urine Obtained after Oral Administration of ^{14}C -TNT (5 mg/kg). Urine was incubated with acetate buffer (pH 5.0) for 24 hr then extracted with ethyl acetate.

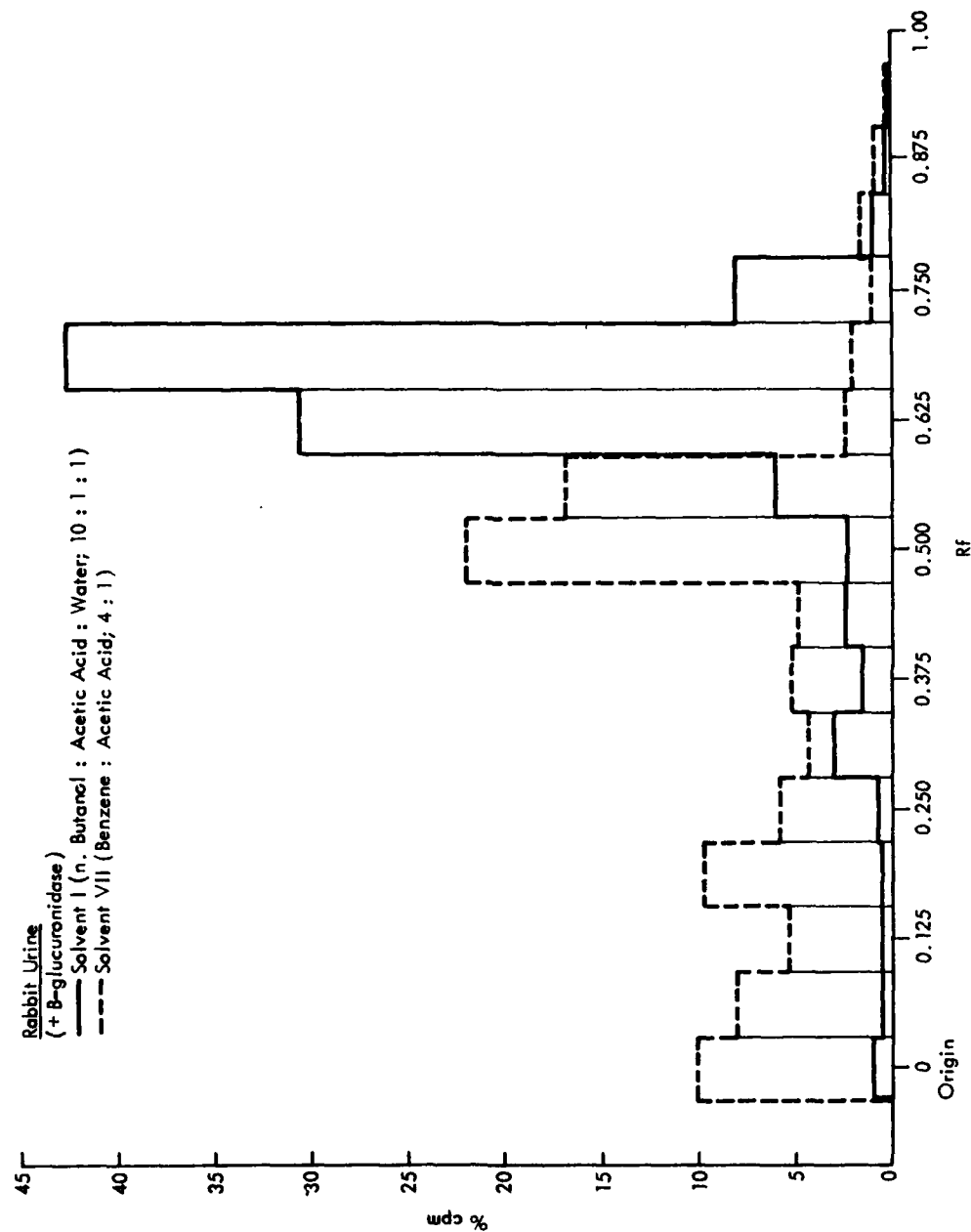


Figure 10-b: TLC of Rabbit Urine Obtained after Oral Administration of ^{14}C -TNT (5 mg/kg).
 Urine was incubated with acetate buffer (pH 5.0) and β -glucuronidase for
 24 hr then extracted with ethyl acetate.

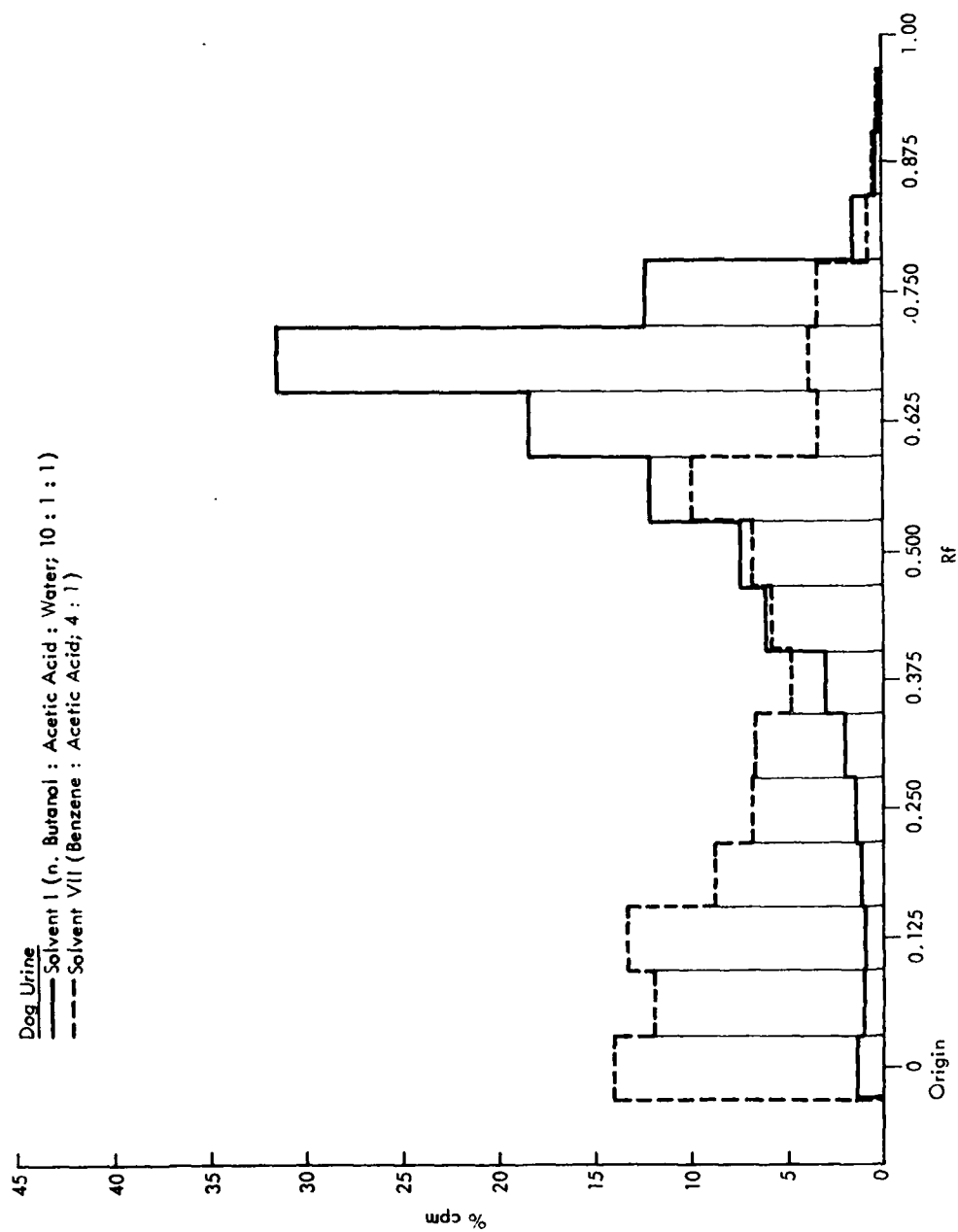


Figure 11-a: TLC of Dog Urine Obtained after Oral Administration of ^{14}C -TNT (5 mg/kg).
Urine was incubated with acetate buffer (pH 5.0) for 24 hr then extracted with ethyl acetate.

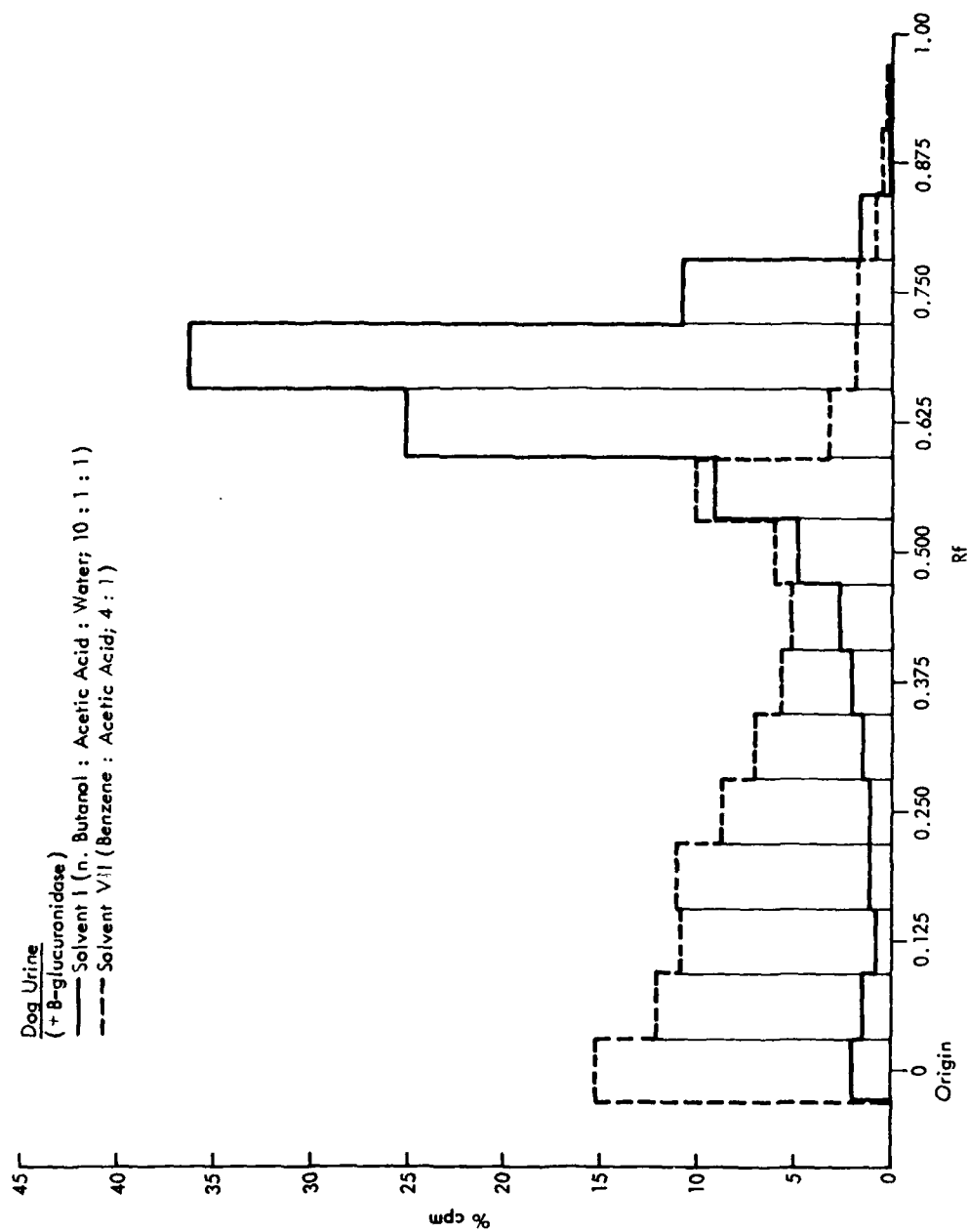


Figure 11-b: TLC of Dog Urine Obtained after Oral Administration of ^{14}C -TNT (5 mg/kg).
 Urine was incubated with acetate buffer (pH 5.0) and β -glucuronidase for 24 hr then extracted with ethyl acetate.

Figure 12: TLC of Raw Urine Obtained from Rats and Mice Treated Orally, Dermally or Intratracheally with ^{14}C -TNT. The TLC plates were developed in two solvent systems: I, n-butanol:acetic acid:water, 10:1:1; IX, toluene:acetic acid, 4:1. Samples of TNT and reference standards (Nos. 1-10, Table 19) were spotted and developed with the same solvents. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 12 follows

SOLVENT 1 NO 159

50.0	0.0000	3135.3	6.0
49.0	.0938	2782.4	5.0
48.0	.1563	4396.5	7.9
47.0	.2188	7939.4	14.3
46.0	.2813	2926.7	5.3
45.0	.3438	3468.2	6.2
44.0	.4063	4955.7	8.9
43.0	.4688	5728.4	10.3
42.0	.5313	8172.5	14.7
41.0	.5938	5113.8	9.2
40.0	.6563	3531.5	6.4
39.0	.7188	2771.7	5.0
38.0	.7813	420.9	.8
37.0	.8438	17.5	.0
36.0	.9063	5.7	.0
35.0	.9688	5.4	.0
34.0	HF		
33.0	0.0000	11.0	
32.0	.2188	27.5	
31.0	.5313	61.5	

P E K C E N T

R A D I O A C T I V I T Y

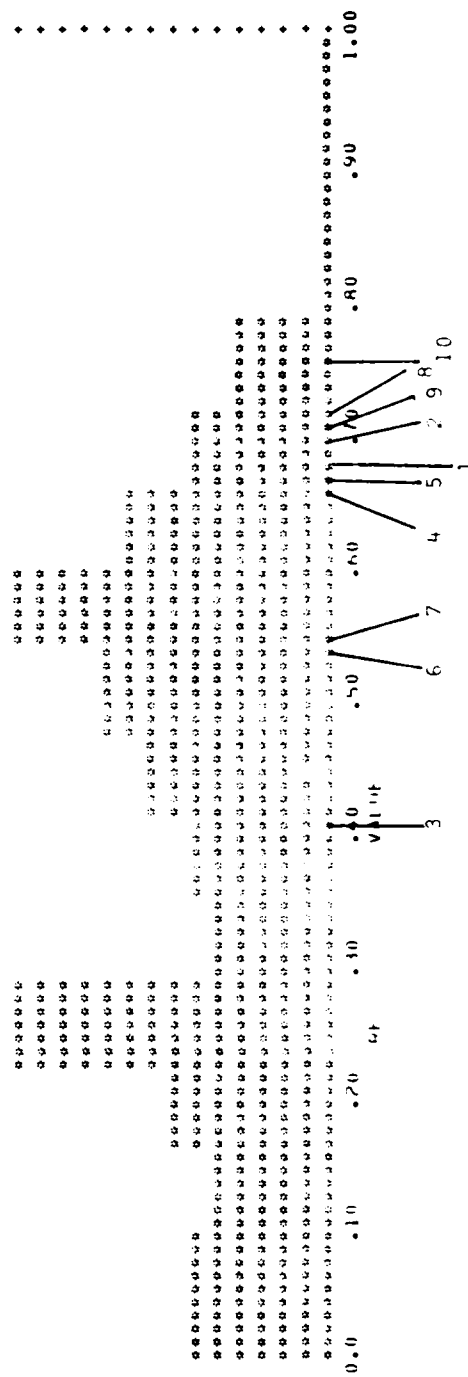


Figure 12-a-1: 24-Hr Urine, Male Rats, Oral Treatment, Solvent 1

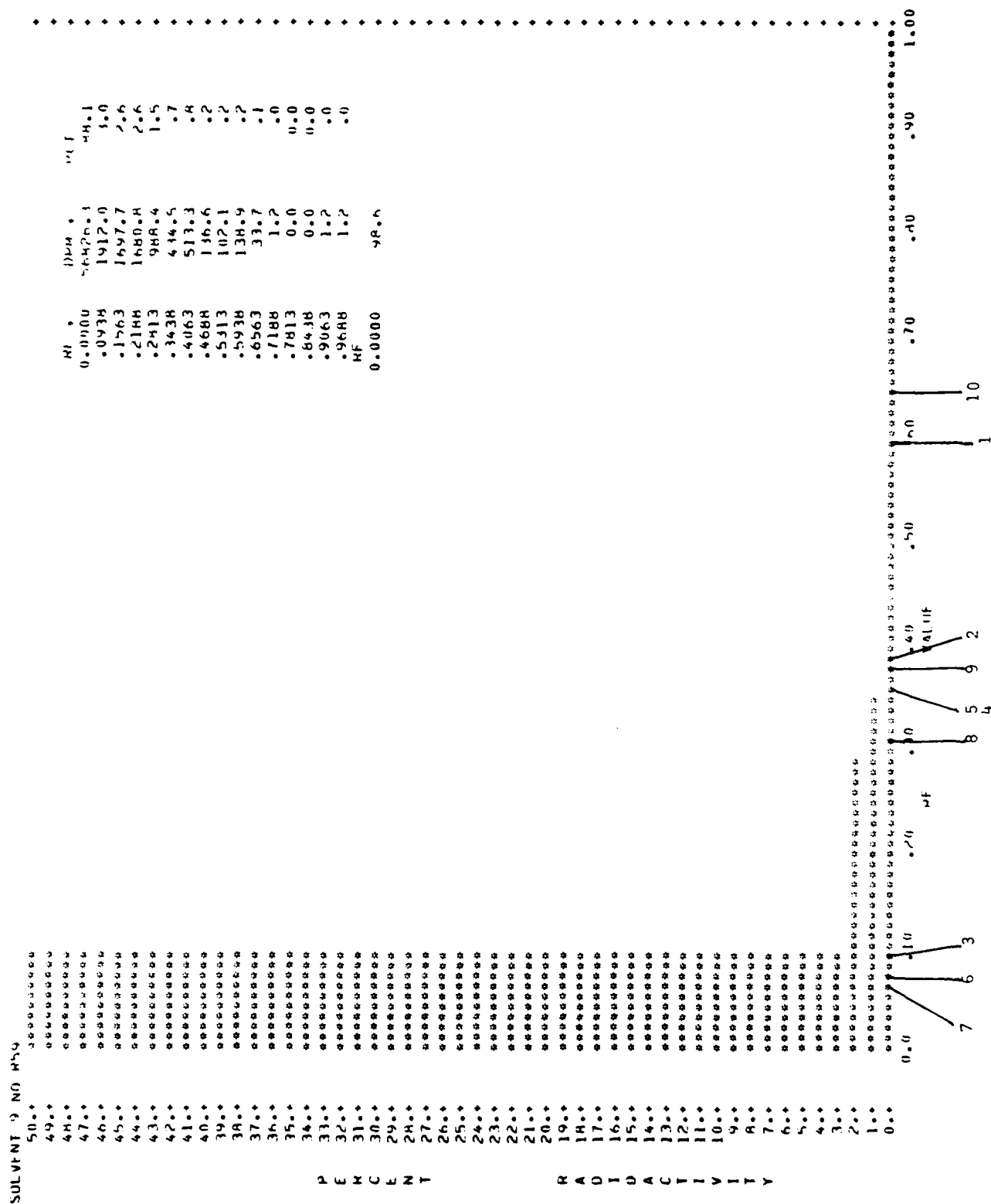


Figure 12-a-IX: 24-Hr Urine, Male Rats, Oral Treatment, Solvent IX

SOLVENT 1 NO H60

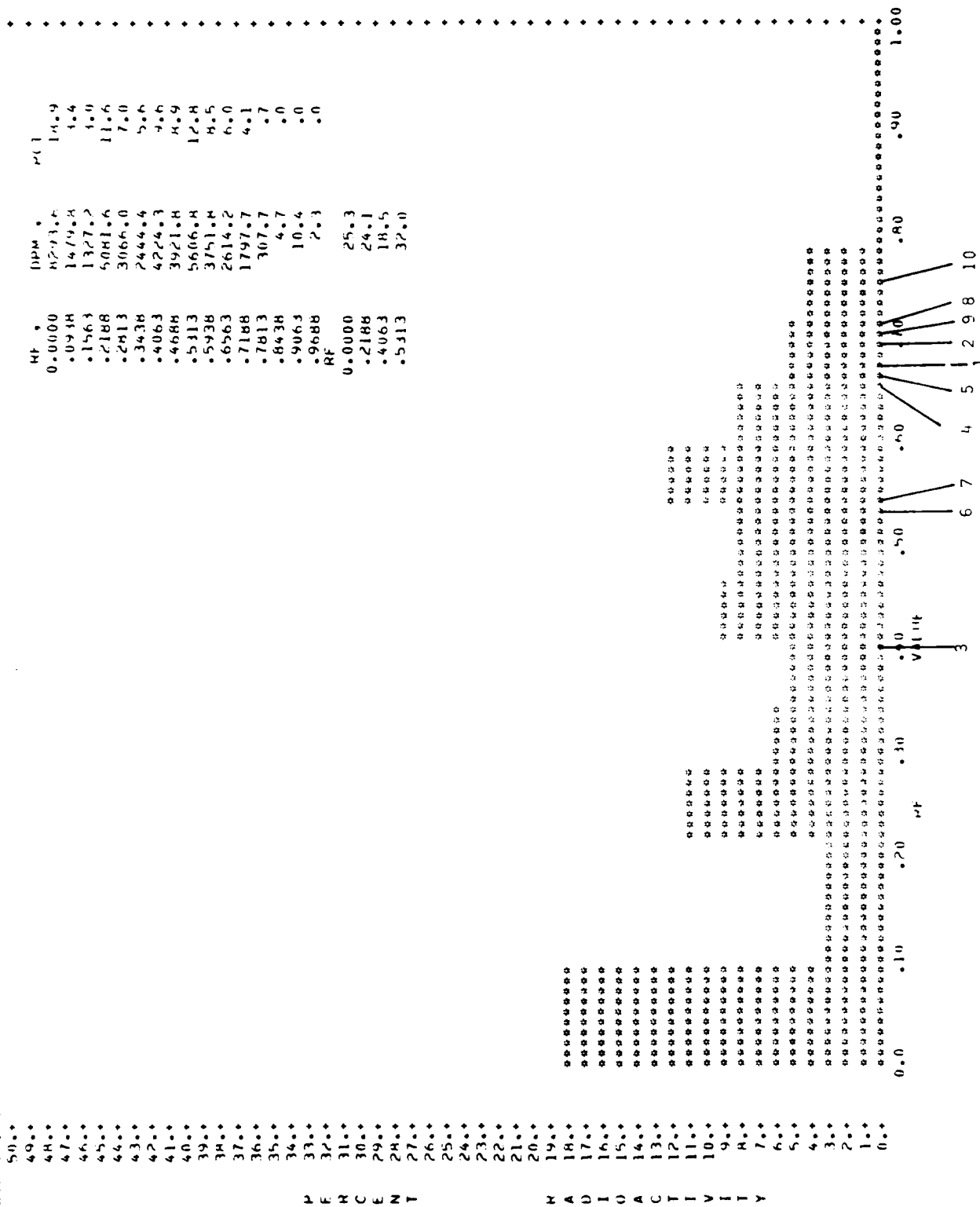
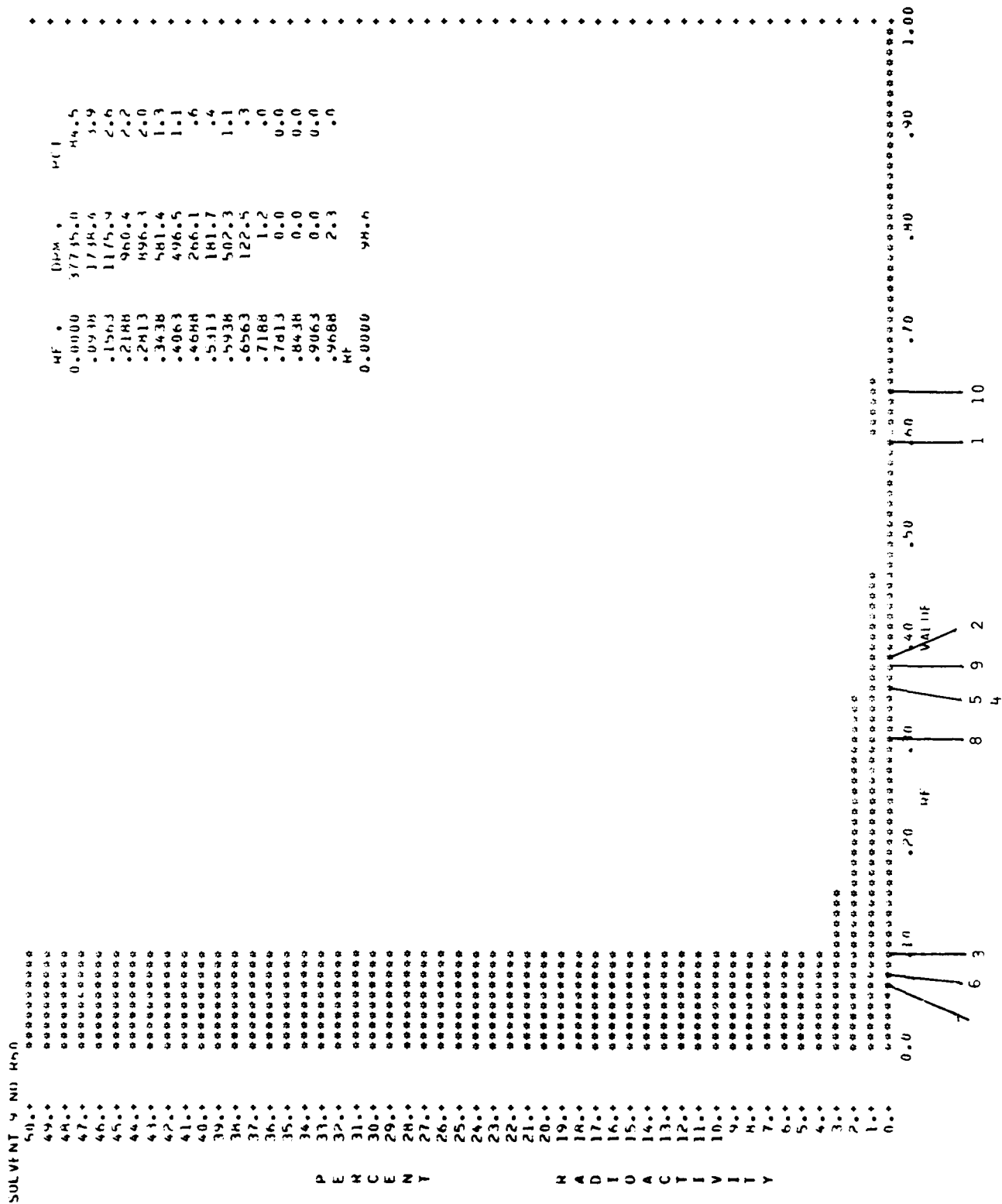


Figure 12-b-I: 24-Hr Urine, Female Rats, Oral Treatment, Solvent I



SOLVENT 1 NO 457

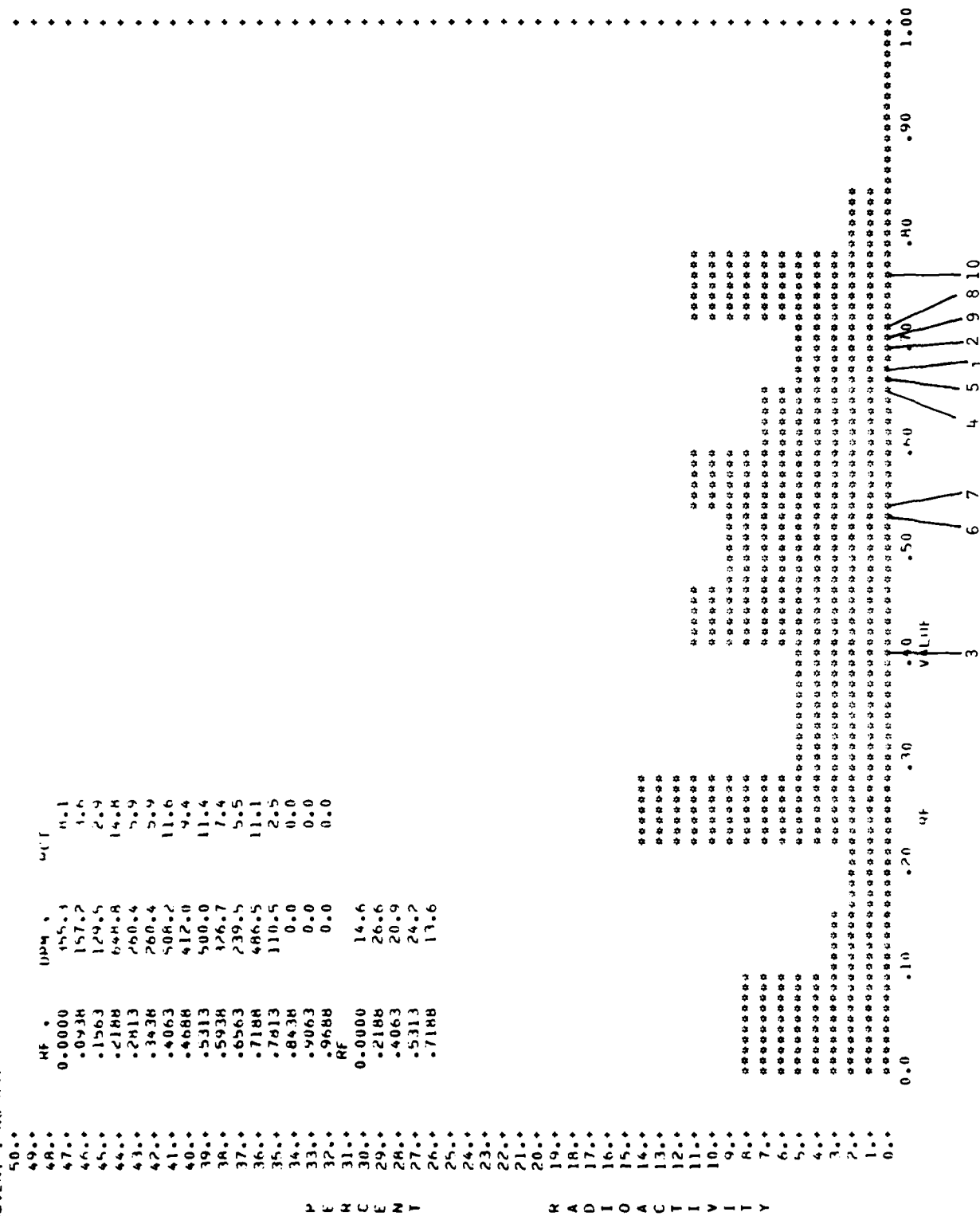
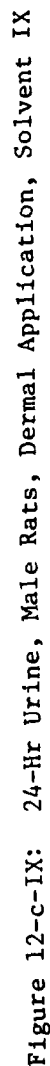


Figure 12-c-I: 24-Hr Urine, Male Rats, Dermal Application, Solvent I



SOLVENT 1 NO R62

50.0	0.0000	1508.7	22.5
49.0	0.038	372.4	5.6
48.0	.1563	216.4	3.2
47.0	.2188	360.0	5.4
46.0	.2813	549.0	4.2
45.0	.3438	347.7	6.0
44.0	.4063	447.4	6.7
43.0	.4688	610.4	9.1
42.0	.5313	626.2	7.4
41.0	.5938	427.7	6.4
40.0	.6563	532.4	4.0
39.0	.7188	528.3	7.9
38.0	.7813	107.2	1.5
37.0	.8438	0.0	0.0
36.0	.9063	0.0	0.0
35.0	.9688	0.0	0.0
34.0	HF		
33.0	0.0000	31.4	
32.0	.2813	19.5	
31.0	.5313	31.6	
30.0	.6563	17.5	

P E H C E N T

R A D I O A C T I V I T Y

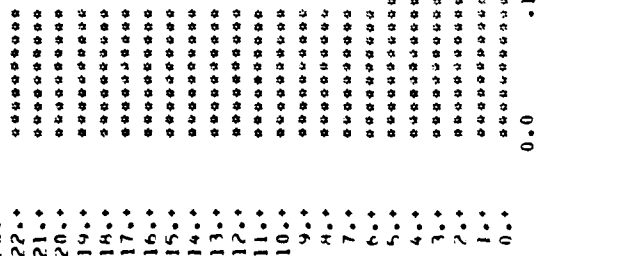


Figure 12-d-I: 24-Hr Urine, Female Rats, Dermal Application, Solvent I

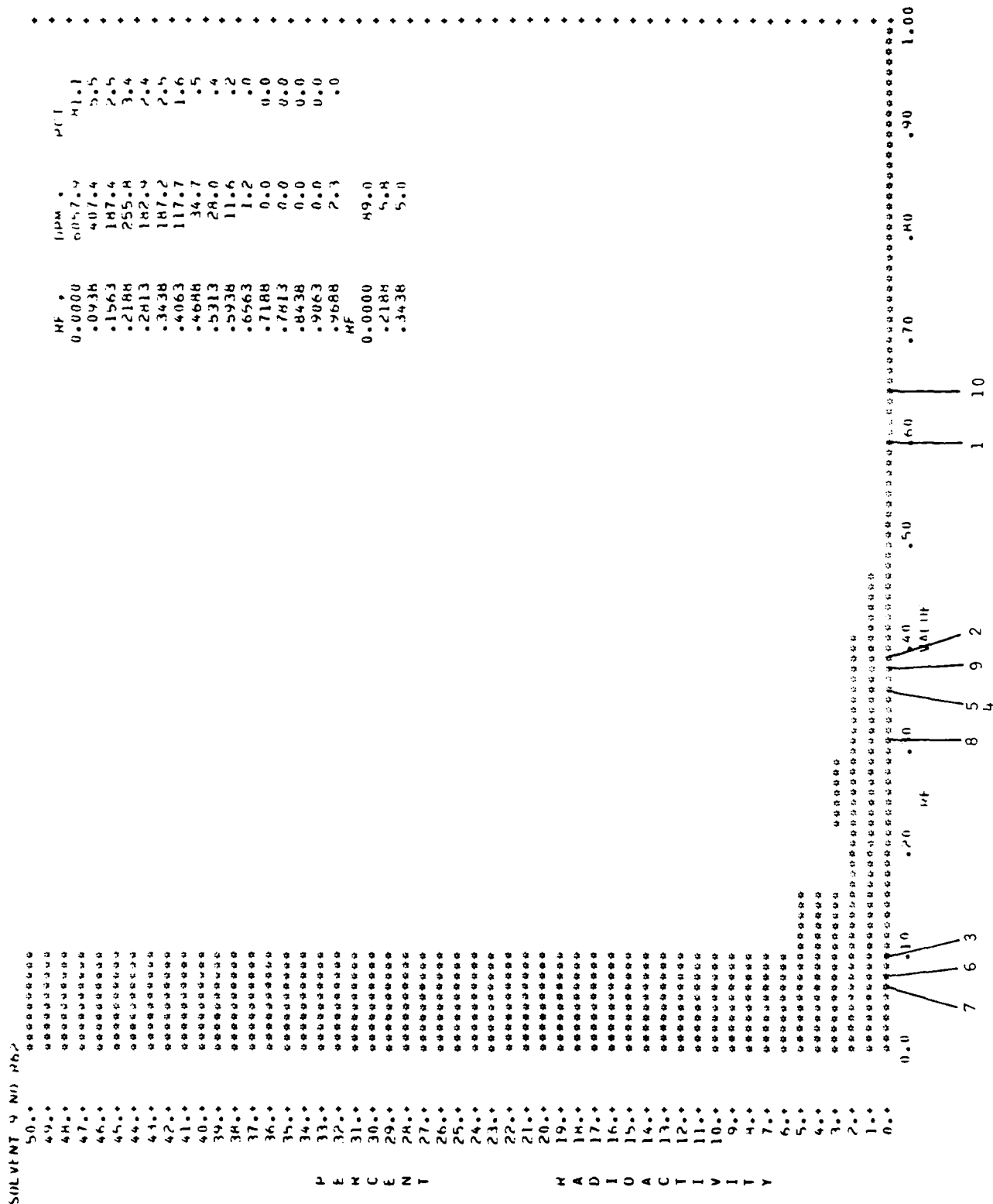


Figure 12-d-IX: 24-Hr Urine, Female Rats, Dermal Application, Solvent IX

SOLVENT I NO R10

50.0	0.0000	1746.5	12.9
49.0	0.0438	308.0	2.3
48.0	0.1563	374.6	2.8
47.0	0.2188	2018.5	14.9
46.0	0.2813	638.4	4.7
45.0	0.3438	536.0	4.0
44.0	0.4063	1347.2	9.9
43.0	0.4688	1081.4	8.0
42.0	0.5313	1373.3	10.1
41.0	0.5938	1913.3	14.1
40.0	0.6563	1183.7	8.7
39.0	0.7188	819.3	6.0
38.0	0.7813	226.1	1.7
37.0	0.8438	1.2	0.0
36.0	0.9063	1.1	0.0
35.0	0.9688	0.0	0.0
34.0	0.0000	15.1	
33.0	0.2188	26.3	
32.0	0.4063	17.9	
31.0	0.5938	40.7	

P E M C E N T

R A D I O A C T I V I T Y

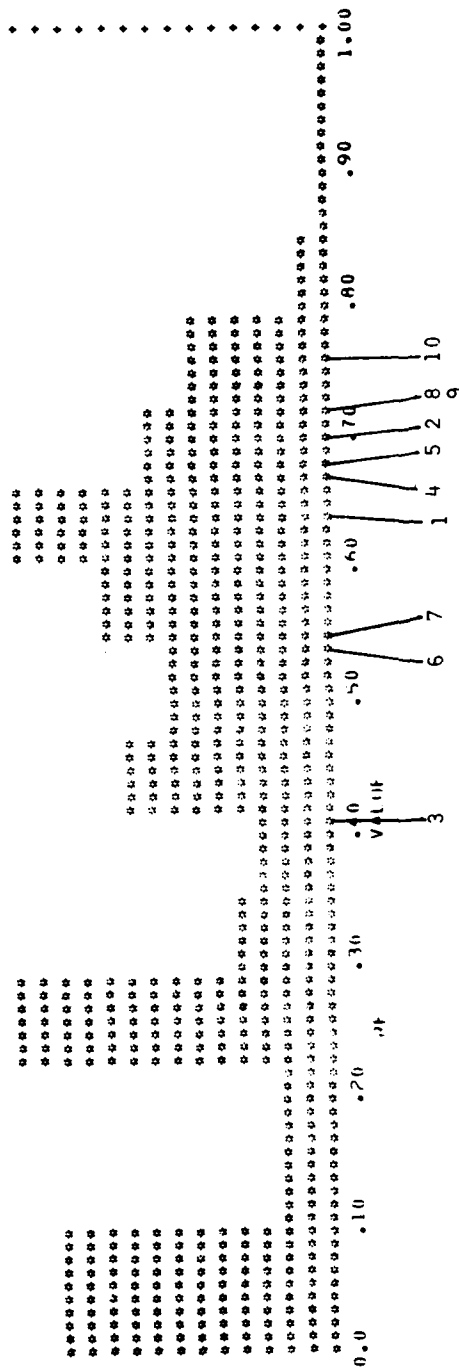


Figure 12-e-I: 4-Hr Urine, Male Rats, Oral Treatment, Solvent I

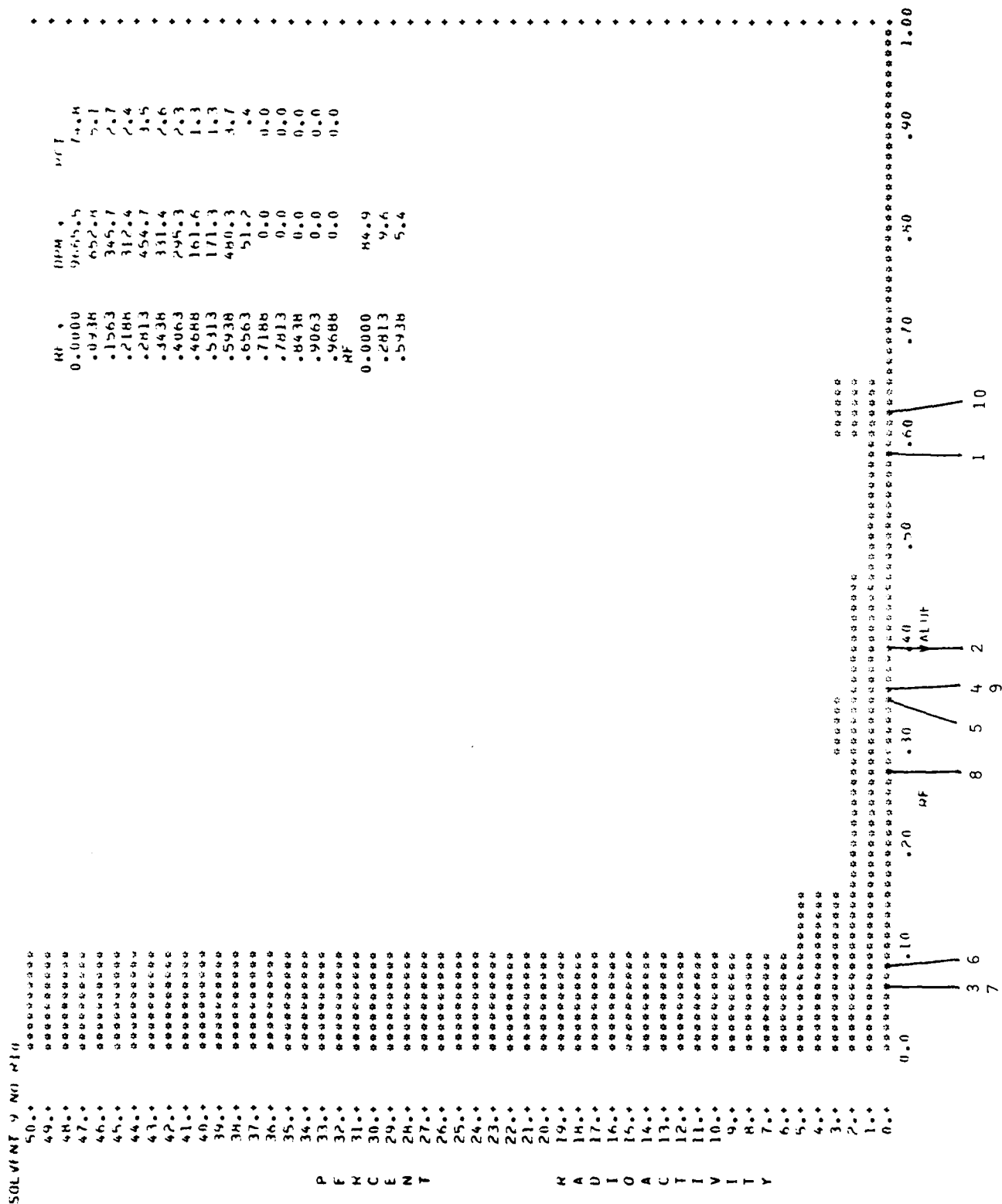
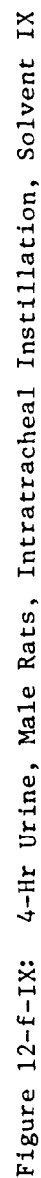


Figure 12-e-IX: 4-Hr Urine, Male Rats, Oral Treatment, Solvent IX



SOLVENT 1 40 MS.6

50.0	0.0000	50022.0	24.4
48.0	.0938	10816.0	5.3
47.0	.1563	14562.5	6.6
46.0	.2188	25461.3	12.4
45.0	.2813	15376.5	7.5
44.0	.3438	10115.4	4.9
43.0	.4063	12872.7	6.3
42.0	.4688	13987.0	6.8
41.0	.5313	14535.0	7.1
40.0	.5938	17433.7	8.5
39.0	.6563	8491.9	4.1
38.0	.7188	9476.8	4.6
37.0	.7813	2474.4	1.2
36.0	.8438	38.2	.0
35.0	.9063	5.8	.0
34.0	.9688	7.0	.0
33.0	0.0000	29.7	
32.0	.2188	31.5	
31.0	.5938	32.9	
30.0	.7188	5.9	

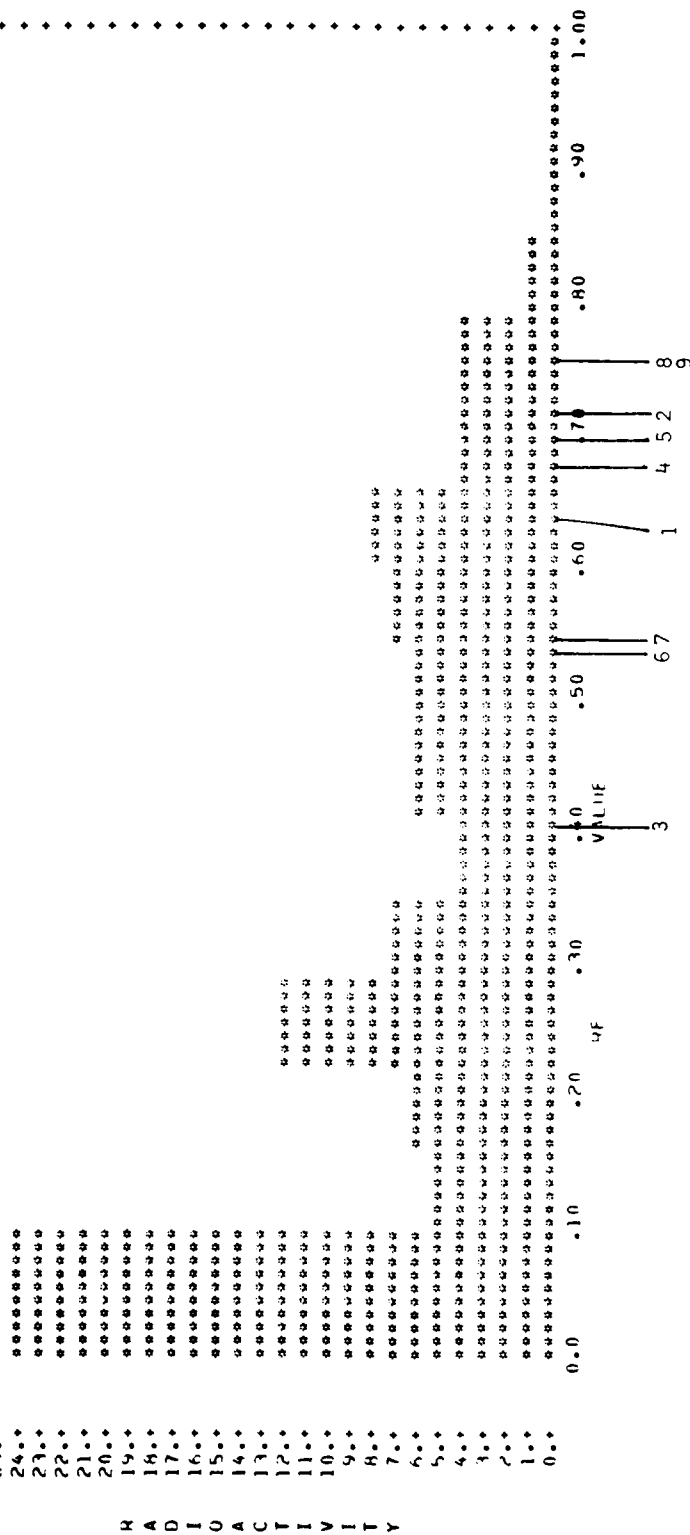


Figure 12-g-I: 24-Hr Urine, Male Mice, Oral Treatment, Solvent I

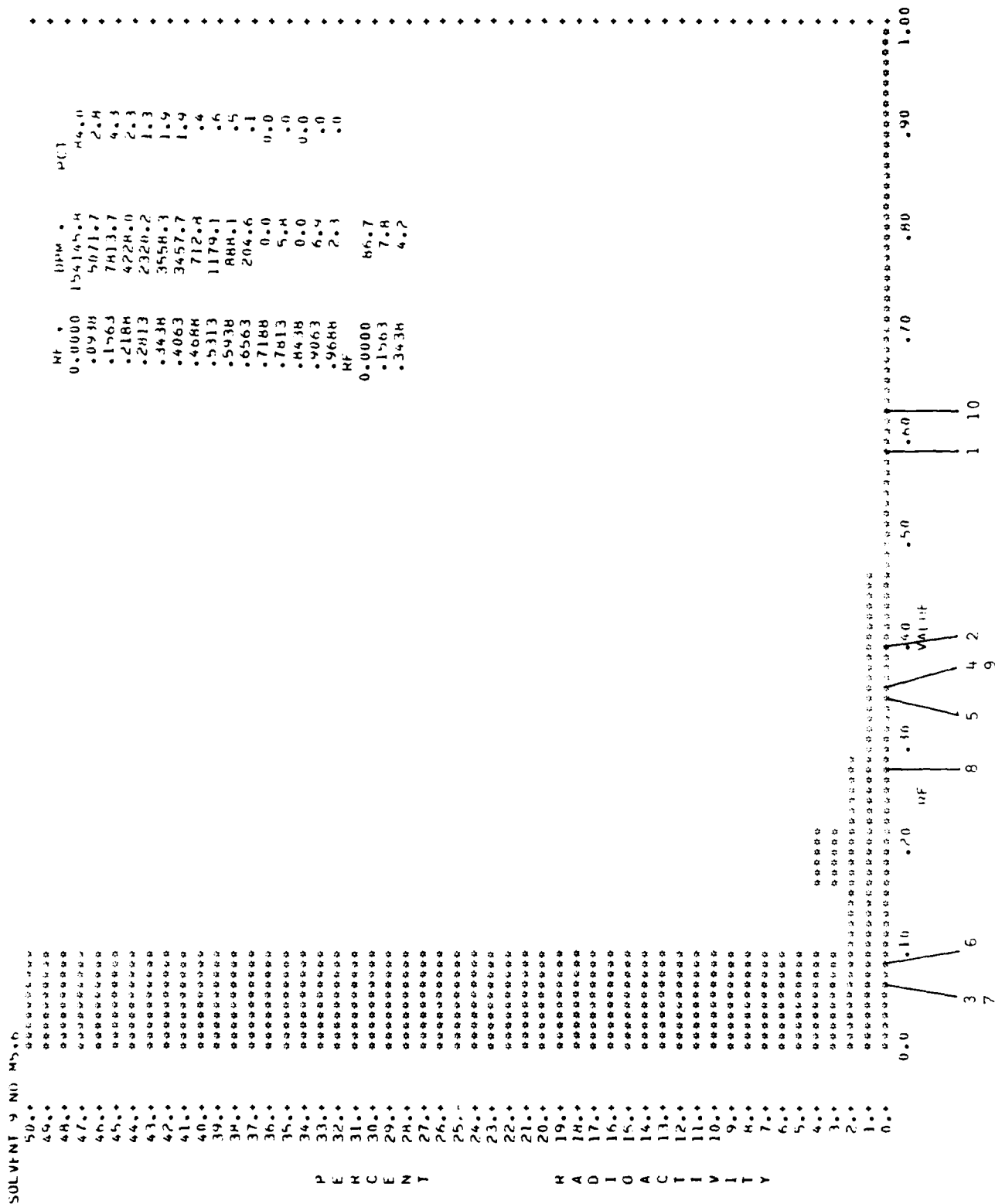


Figure 12-g-IX: 24-Hr Urine, Male Mice, Oral Treatment, Solvent IX

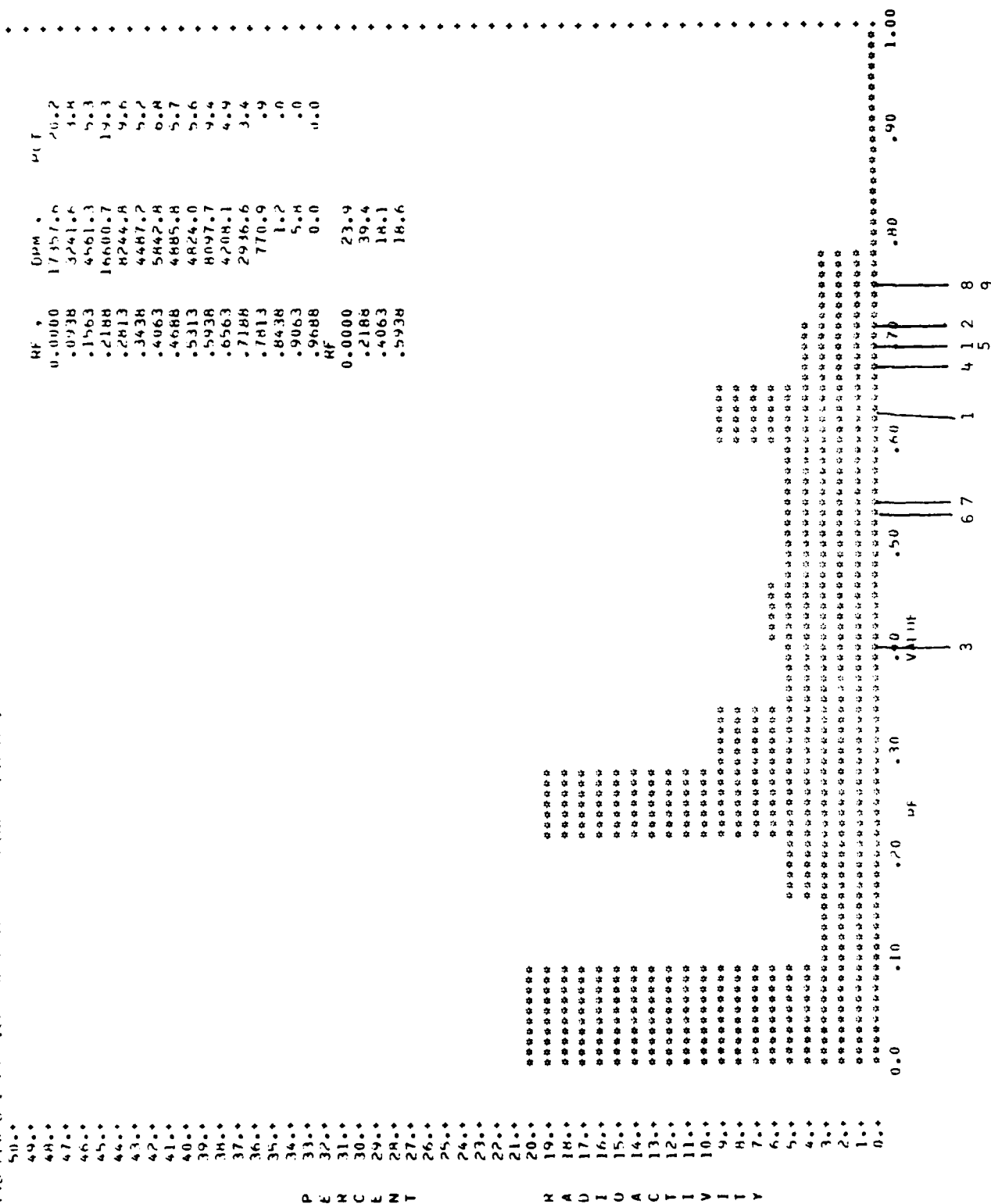


Figure 12-h-I: 24-Hr Urine, Male Mice, Dermal Application, Solvent I

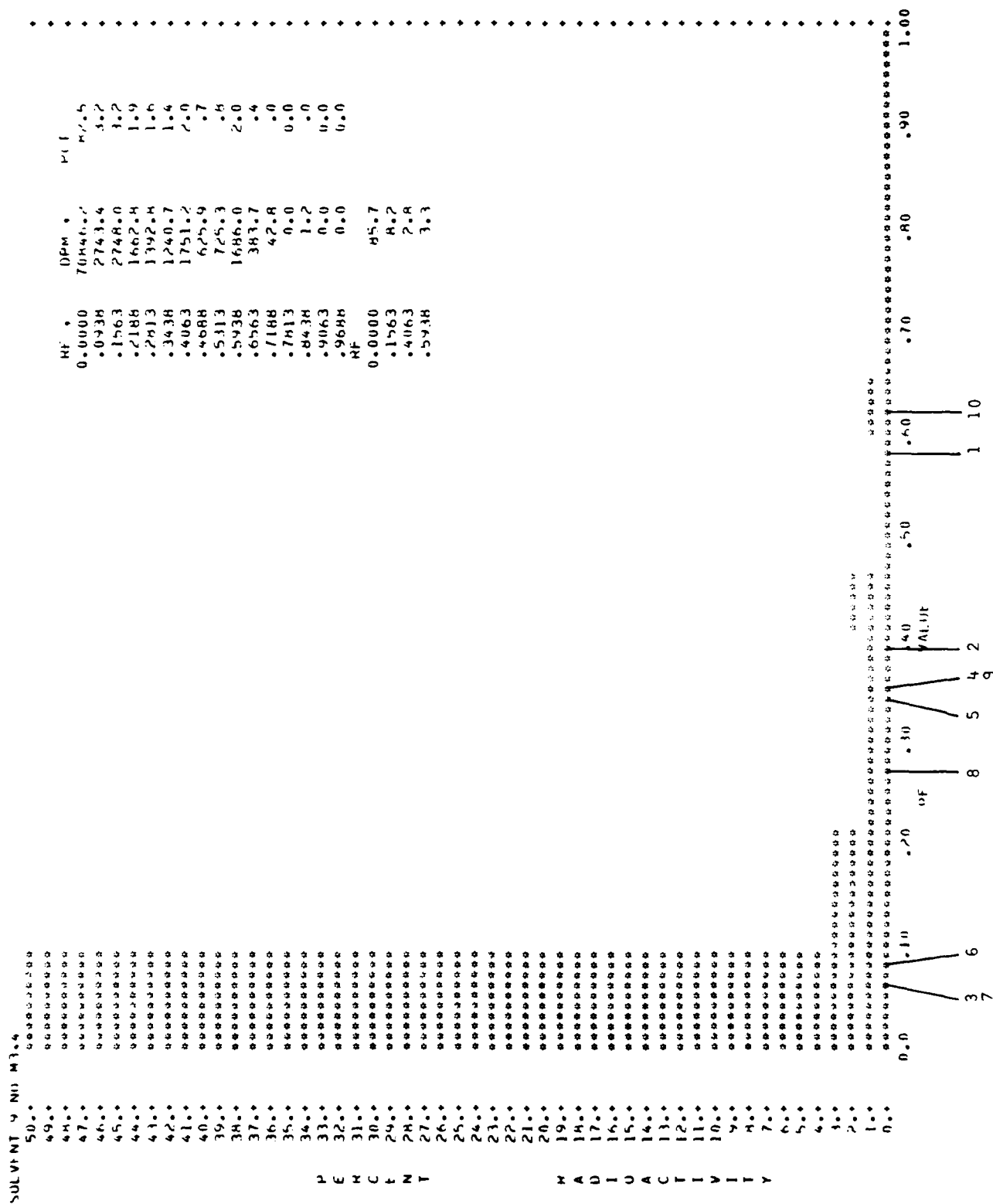


Figure 12-h-IX: 24 Hr Urine, Male Mice, Dermal Application, Solvent IX

Figure 13: TLC of Lyophilized Urine Obtained from Rats, Mice and Rabbits Treated Orally or Dermally with ^{14}C -TNT. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 13 follows

	RF	Diff	PCI
50.0	0.0000	52.4	1.6
49.0	.0438	26.6	1.3
48.0	.1563	24.0	1.4
47.0	.2188	54.1	1.9
46.0	.2813	186.1	9.2
45.0	.3438	140.0	7.9
44.0	.4063	341.0	16.9
43.0	.4688	335.7	16.6
42.0	.5313	274.9	11.8
41.0	.5938	223.3	11.0
40.0	.6563	302.6	16.9
39.0	.7188	50.1	2.5
38.0	.7813	2.3	.1
37.0	.8438	0.0	0.0
36.0	.9063	0.0	0.0
35.0	.9688	0.0	0.0
34.0	RF	%	
33.0	0.0000	3.9	
32.0	.2813	20.3	
31.0	.4063	54.1	
30.0	.6563	17.5	

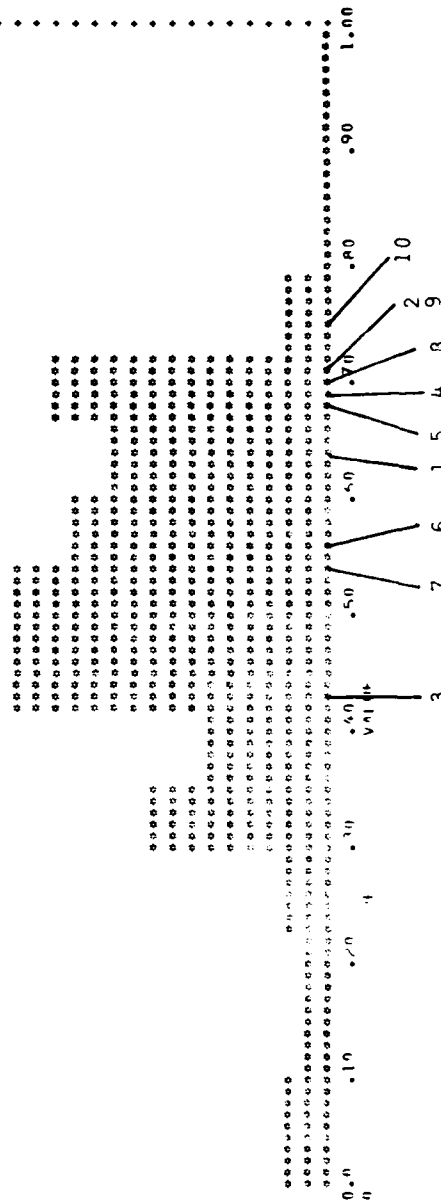


Figure 13-a-I: Male Rats, Oral Treatment, Solvent I

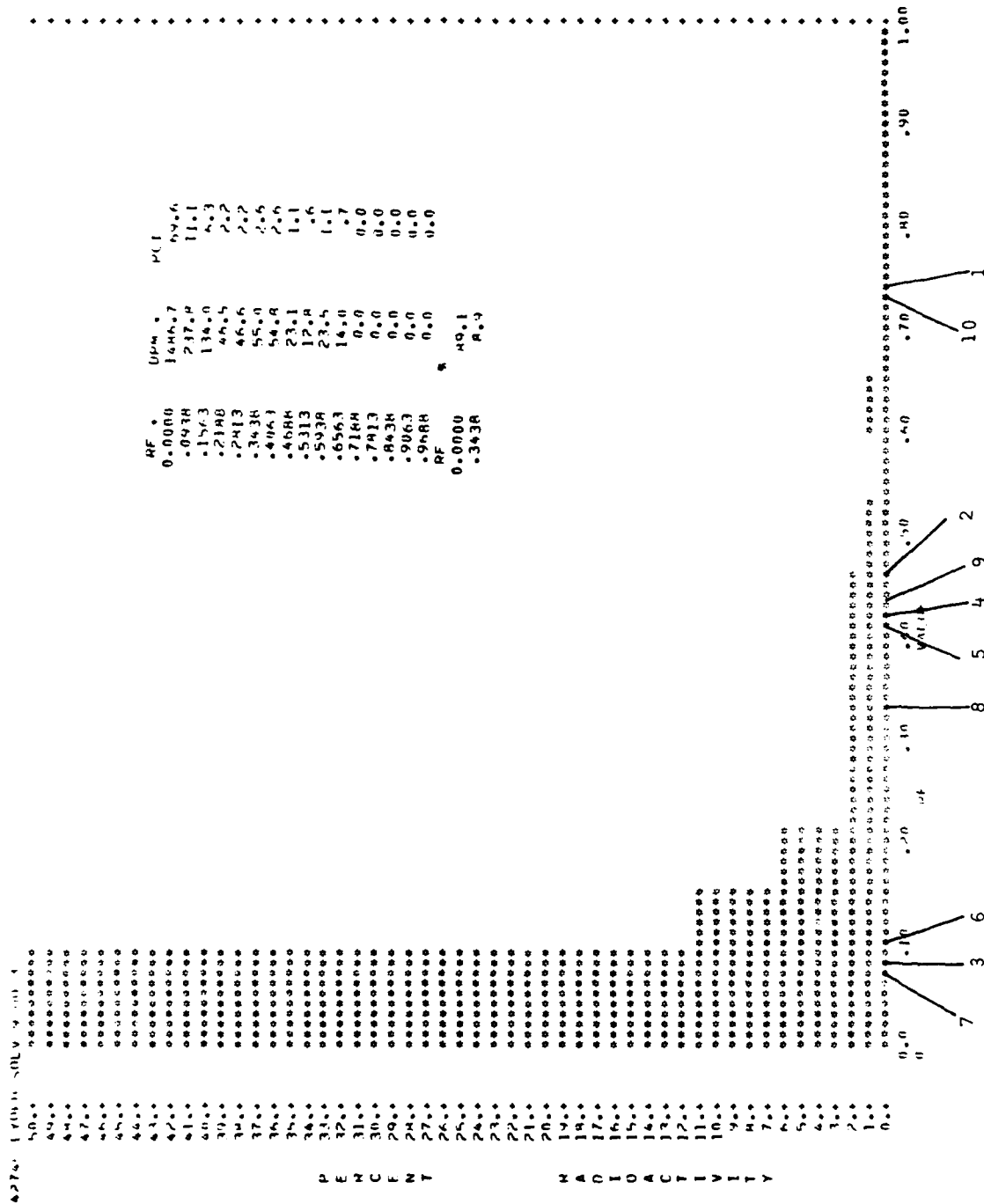


Figure 13-a-IX: Male Rats, Oral Treatment, Solvent IX

42768 LYOPH SOLV 1.00

50.0	0.0000	43.0	1.5
49.0	.0038	40.0	1.7
48.0	.1503	35.0	2.4
47.0	.2184	34.5	3.2
46.0	.2413	35.7	7.0
45.0	.3436	33.4	10.9
44.0	.4063	34.6	15.3
43.0	.4694	226.9	14.4
42.0	.5313	142.0	14.8
41.0	.5934	110.7	9.0
40.0	.6563	117.4	9.6
39.0	.7188	45.4	4.7
38.0	.7813	0.0	0.0
37.0	.8438	0.0	0.0
36.0	.9063	0.0	0.0
35.0	.9688	0.0	0.0
34.0	MF	5.1	
33.0	0.0000	41.1	
32.0	.4688	13.2	
31.0	.6563		

P E H C E N T

H A N I O A C T I V I T Y

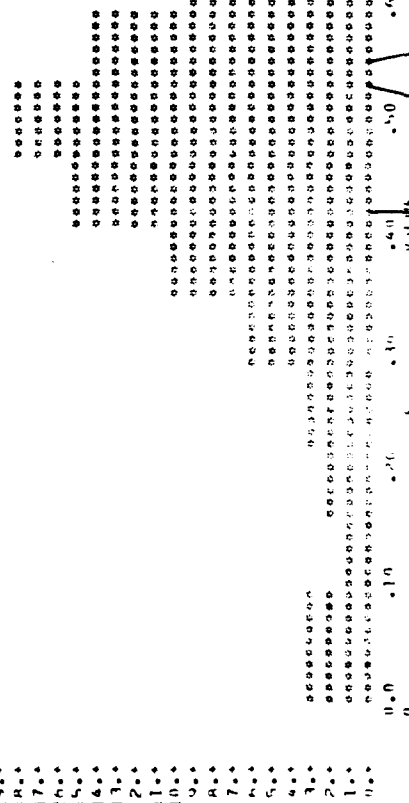


Figure 13-b-I: Female Rats, Oral Treatment, Solvent I

427.60 LYOPH SOLV 1 40 2 100 21

50.0	RF	100.0	100.0
49.0	0.0000	100.0	100.0
48.0	0.0000	100.0	100.0
47.0	0.0000	100.0	100.0
46.0	0.0000	100.0	100.0
45.0	0.0000	100.0	100.0
44.0	0.0000	100.0	100.0
43.0	0.0000	100.0	100.0
42.0	0.0000	100.0	100.0
41.0	0.0000	100.0	100.0
40.0	0.0000	100.0	100.0
39.0	0.0000	100.0	100.0
38.0	0.0000	100.0	100.0
37.0	0.0000	100.0	100.0
36.0	0.0000	100.0	100.0
35.0	0.0000	100.0	100.0
34.0	0.0000	100.0	100.0
33.0	0.0000	100.0	100.0
32.0	0.0000	100.0	100.0
31.0	0.0000	100.0	100.0
30.0	0.0000	100.0	100.0
29.0	0.0000	100.0	100.0
28.0	0.0000	100.0	100.0
27.0	0.0000	100.0	100.0
26.0	0.0000	100.0	100.0
25.0	0.0000	100.0	100.0
24.0	0.0000	100.0	100.0
23.0	0.0000	100.0	100.0
22.0	0.0000	100.0	100.0
21.0	0.0000	100.0	100.0
20.0	0.0000	100.0	100.0
19.0	0.0000	100.0	100.0
18.0	0.0000	100.0	100.0
17.0	0.0000	100.0	100.0
16.0	0.0000	100.0	100.0
15.0	0.0000	100.0	100.0
14.0	0.0000	100.0	100.0
13.0	0.0000	100.0	100.0
12.0	0.0000	100.0	100.0
11.0	0.0000	100.0	100.0
10.0	0.0000	100.0	100.0
9.0	0.0000	100.0	100.0
8.0	0.0000	100.0	100.0
7.0	0.0000	100.0	100.0
6.0	0.0000	100.0	100.0
5.0	0.0000	100.0	100.0
4.0	0.0000	100.0	100.0
3.0	0.0000	100.0	100.0
2.0	0.0000	100.0	100.0
1.0	0.0000	100.0	100.0
0.0	0.0000	100.0	100.0

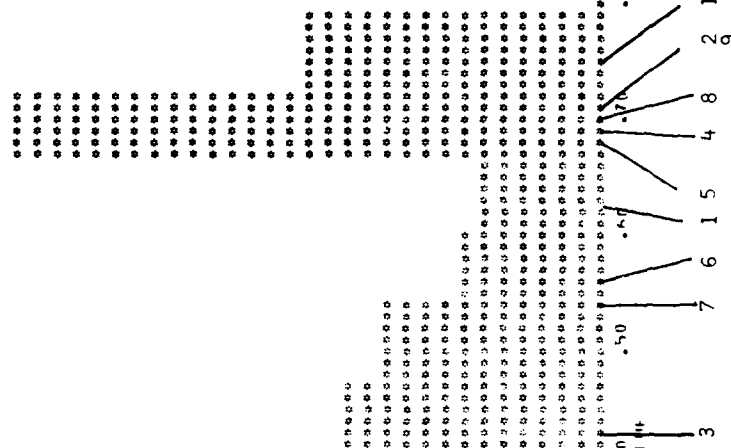


Figure 13-c-I: Male Rats, Dermal Application, Solvent I

42740 LYON-SOLV 9 100

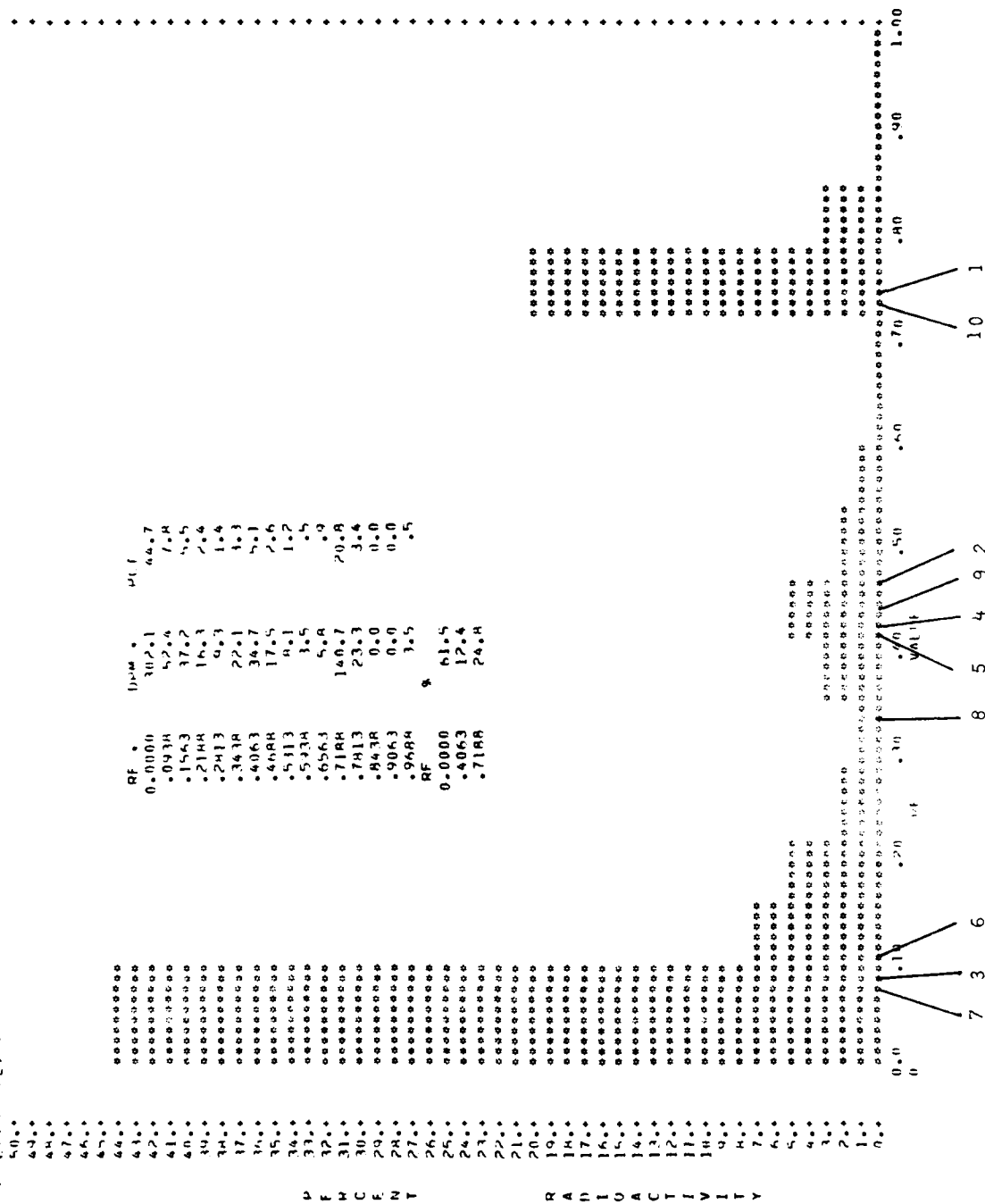


Figure 13-c-IX: Male Rats, Dermal Application, Solvent IX

42740-1 (1000) SOLV 1 1.00

P	50.0	RF	0.0000	1.00	1.1
F	44.0	0.0000	2.0	2.1	2.1
H	47.0	0.0000	2.0	2.1	2.1
R	46.0	0.0000	2.0	2.1	2.1
C	45.0	0.0000	2.0	2.1	2.1
E	44.0	0.0000	2.0	2.1	2.1
N	43.0	0.0000	2.0	2.1	2.1
T	42.0	0.0000	2.0	2.1	2.1
	41.0	0.0000	2.0	2.1	2.1
	40.0	0.0000	2.0	2.1	2.1
	39.0	0.0000	2.0	2.1	2.1
	38.0	0.0000	2.0	2.1	2.1
	37.0	0.0000	2.0	2.1	2.1
	36.0	0.0000	2.0	2.1	2.1
	35.0	0.0000	2.0	2.1	2.1
	34.0	0.0000	2.0	2.1	2.1
	33.0	0.0000	2.0	2.1	2.1
	32.0	0.0000	2.0	2.1	2.1
	31.0	0.0000	2.0	2.1	2.1
	30.0	0.0000	2.0	2.1	2.1
	29.0	0.0000	2.0	2.1	2.1
	28.0	0.0000	2.0	2.1	2.1
	27.0	0.0000	2.0	2.1	2.1
	26.0	0.0000	2.0	2.1	2.1
	25.0	0.0000	2.0	2.1	2.1
	24.0	0.0000	2.0	2.1	2.1
	23.0	0.0000	2.0	2.1	2.1
	22.0	0.0000	2.0	2.1	2.1
	21.0	0.0000	2.0	2.1	2.1
	20.0	0.0000	2.0	2.1	2.1
	19.0	0.0000	2.0	2.1	2.1
	18.0	0.0000	2.0	2.1	2.1
	17.0	0.0000	2.0	2.1	2.1
	16.0	0.0000	2.0	2.1	2.1
	15.0	0.0000	2.0	2.1	2.1
	14.0	0.0000	2.0	2.1	2.1
	13.0	0.0000	2.0	2.1	2.1
	12.0	0.0000	2.0	2.1	2.1
	11.0	0.0000	2.0	2.1	2.1
	10.0	0.0000	2.0	2.1	2.1
	9.0	0.0000	2.0	2.1	2.1
	8.0	0.0000	2.0	2.1	2.1
	7.0	0.0000	2.0	2.1	2.1
	6.0	0.0000	2.0	2.1	2.1
	5.0	0.0000	2.0	2.1	2.1
	4.0	0.0000	2.0	2.1	2.1
	3.0	0.0000	2.0	2.1	2.1
	2.0	0.0000	2.0	2.1	2.1
	1.0	0.0000	2.0	2.1	2.1
	0.0	0.0000	2.0	2.1	2.1

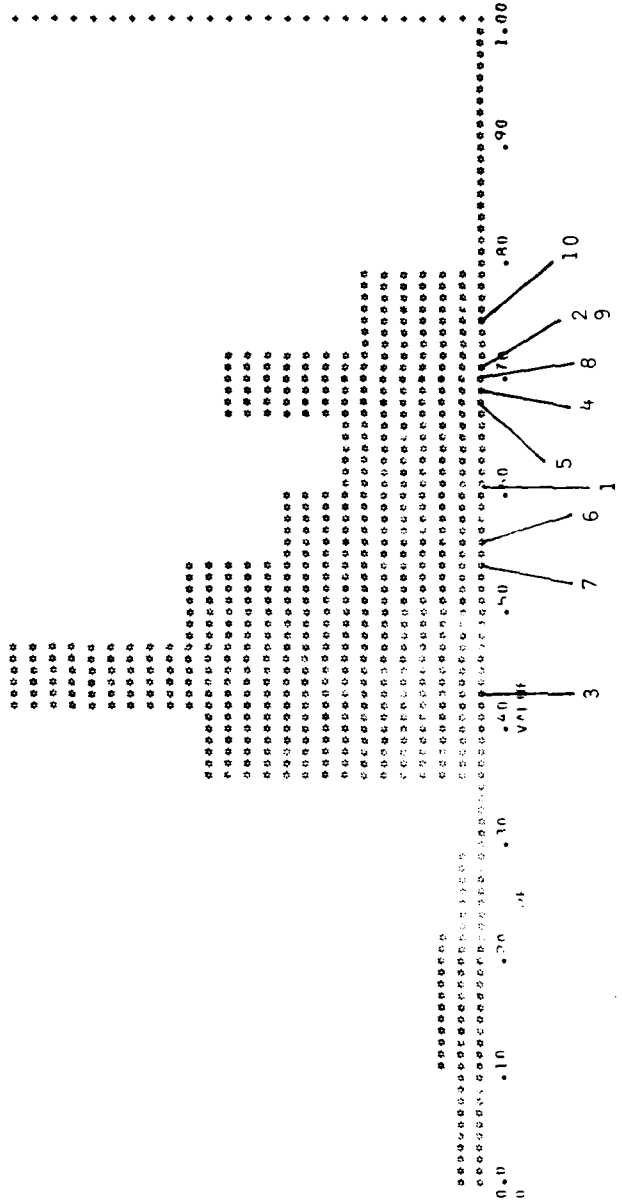


Figure 13-d-I: Female Rats, Dermal Application, Solvent I

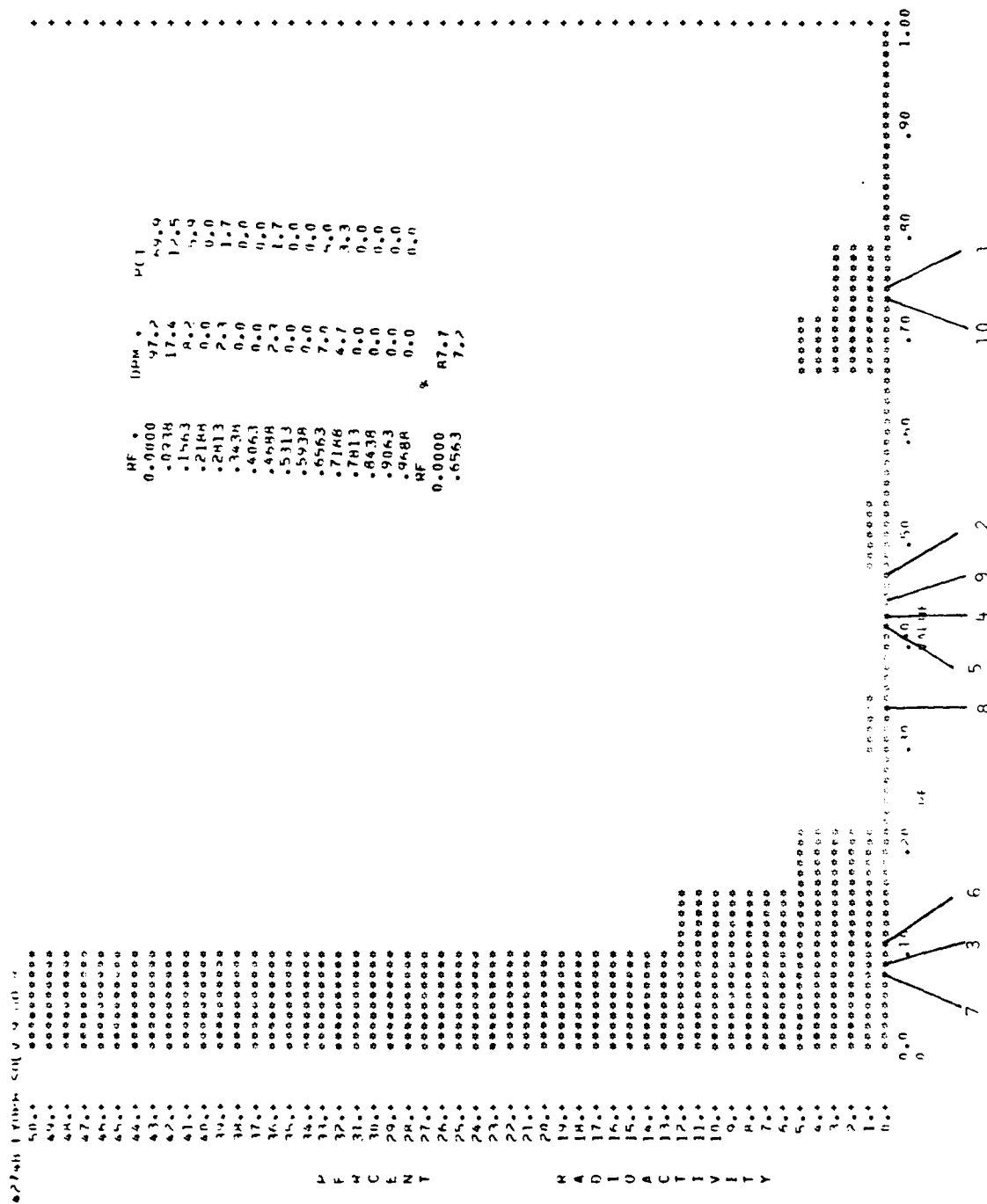


Figure 13-d-IX: Female Rats, Dermal Application, Solvent IX

42744 10000 10000 10000

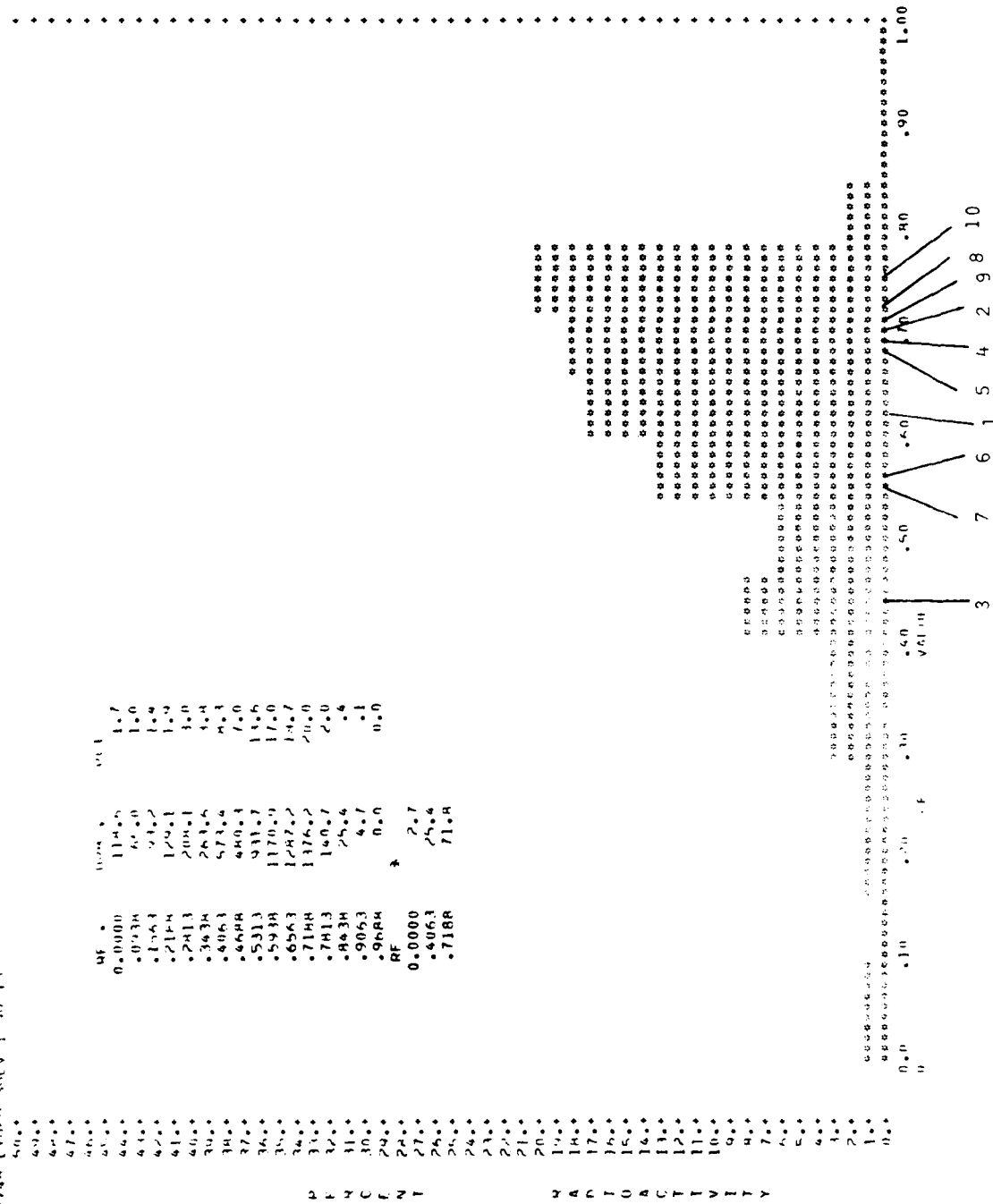


Figure 13-e-I: Male Mice, Oral Treatment, Solvent I

[illegible]

HF	low	PL
0.0000	21492.3	34.4
0.0434	21493.6	12.3
0.1563	411.4	5.7
0.2144	2146.3	4.5
0.2413	2146.3	3.3
0.3434	219.1	3.9
0.4663	996.2	11.4
0.4664	410.4	4.4
0.5113	2332.3	1.3
0.5934	191.7	7.4
0.6563	266.2	6.4
0.7184	19.1	1.1
0.7413	14.4	3
0.8434	0.0	0.0
0.9063	0.0	0.0
0.9664	0.0	0.0
PF		
0.0000	65.6	
0.4063	29.6	
0.6563	4.4	

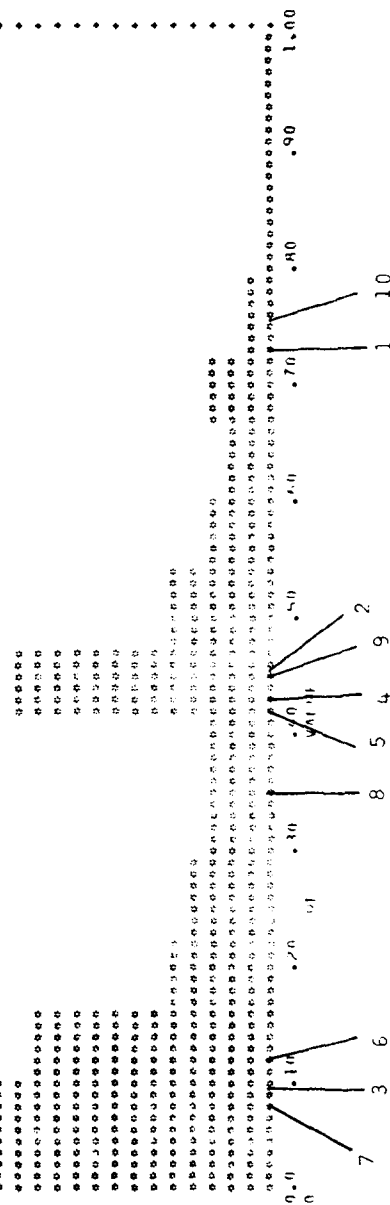


Figure 13-e-IX: Male Mice, Oral Treatment, Solvent IX

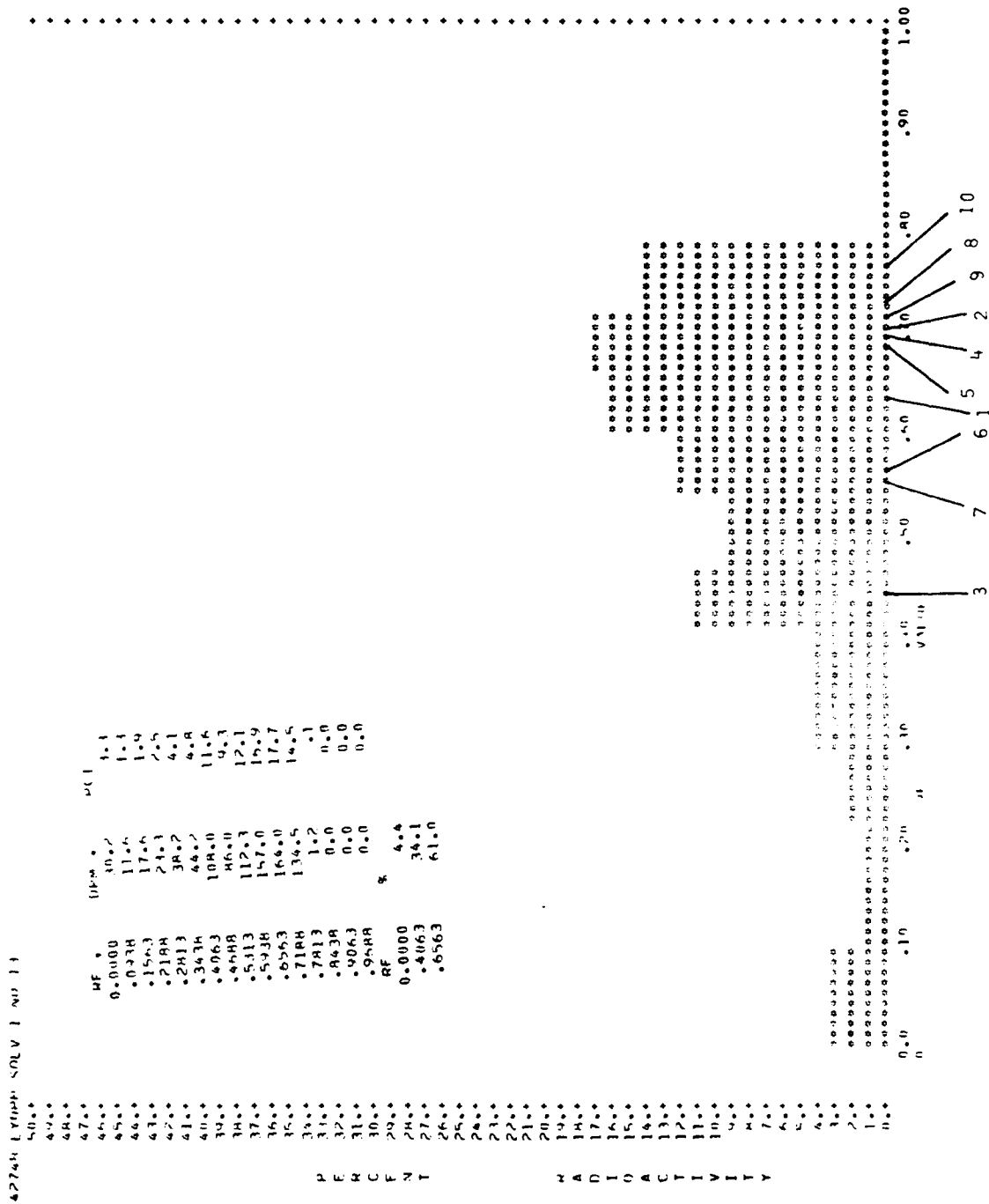


Figure 13-f-I: Male Mice, Dermal Application, Solvent I

4276H LUDPH SOLV 9 100 13

50.0

49.0

48.0

47.0

46.0

45.0

44.0

43.0

42.0

41.0

40.0

39.0

38.0

37.0

36.0

35.0

34.0

33.0

32.0

31.0

30.0

29.0

28.0

27.0

26.0

25.0

24.0

23.0

22.0

21.0

20.0

19.0

18.0

17.0

16.0

15.0

14.0

13.0

12.0

11.0

10.0

9.0

8.0

7.0

6.0

5.0

4.0

3.0

2.0

1.0

0.0

P

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A

C

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I

V

I

Y

Wt

100%

99.1

98.4

97.7

97.0

96.3

95.6

94.9

94.2

93.5

92.8

92.1

91.4

90.7

90.0

89.3

88.6

87.9

87.2

86.5

85.8

85.1

84.4

83.7

83.0

82.3

81.6

80.9

80.2

79.5

78.8

78.1

77.4

76.7

76.0

75.3

74.6

73.9

73.2

72.5

71.8

71.1

70.4

69.7

69.0

68.3

67.6

66.9

66.2

65.5

64.8

64.1

63.4

62.7

62.0

61.3

60.6

59.9

59.2

58.5

57.8

57.1

56.4

55.7

55.0

54.3

53.6

52.9

52.2

51.5

50.8

50.1

49.4

48.7

48.0

1.00

.90

.80

.70

.60

.50

.40

.30

.20

.10

0.00

Figure 13-f-IX: Male Mice, Dermal Application, Solvent IX

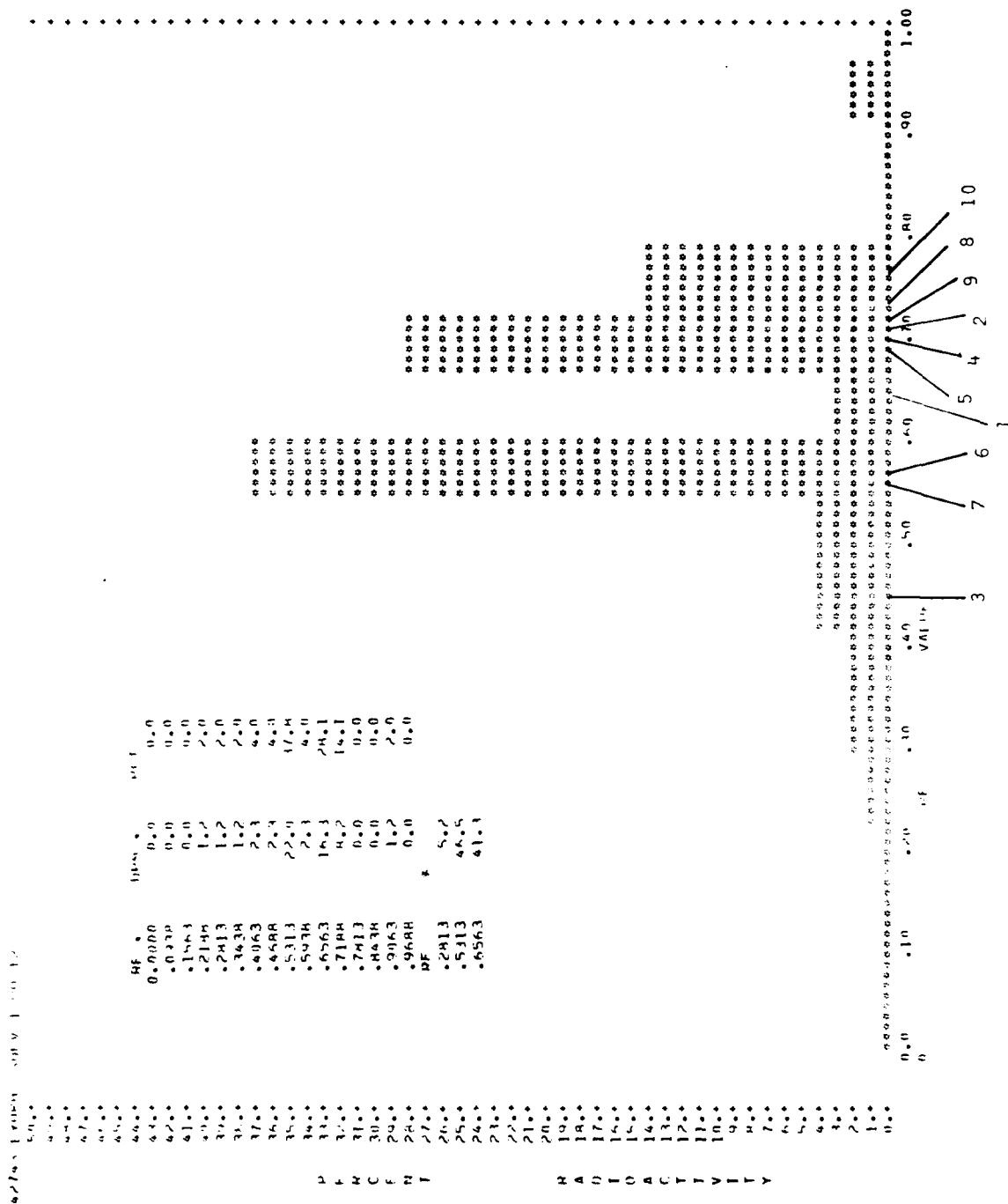
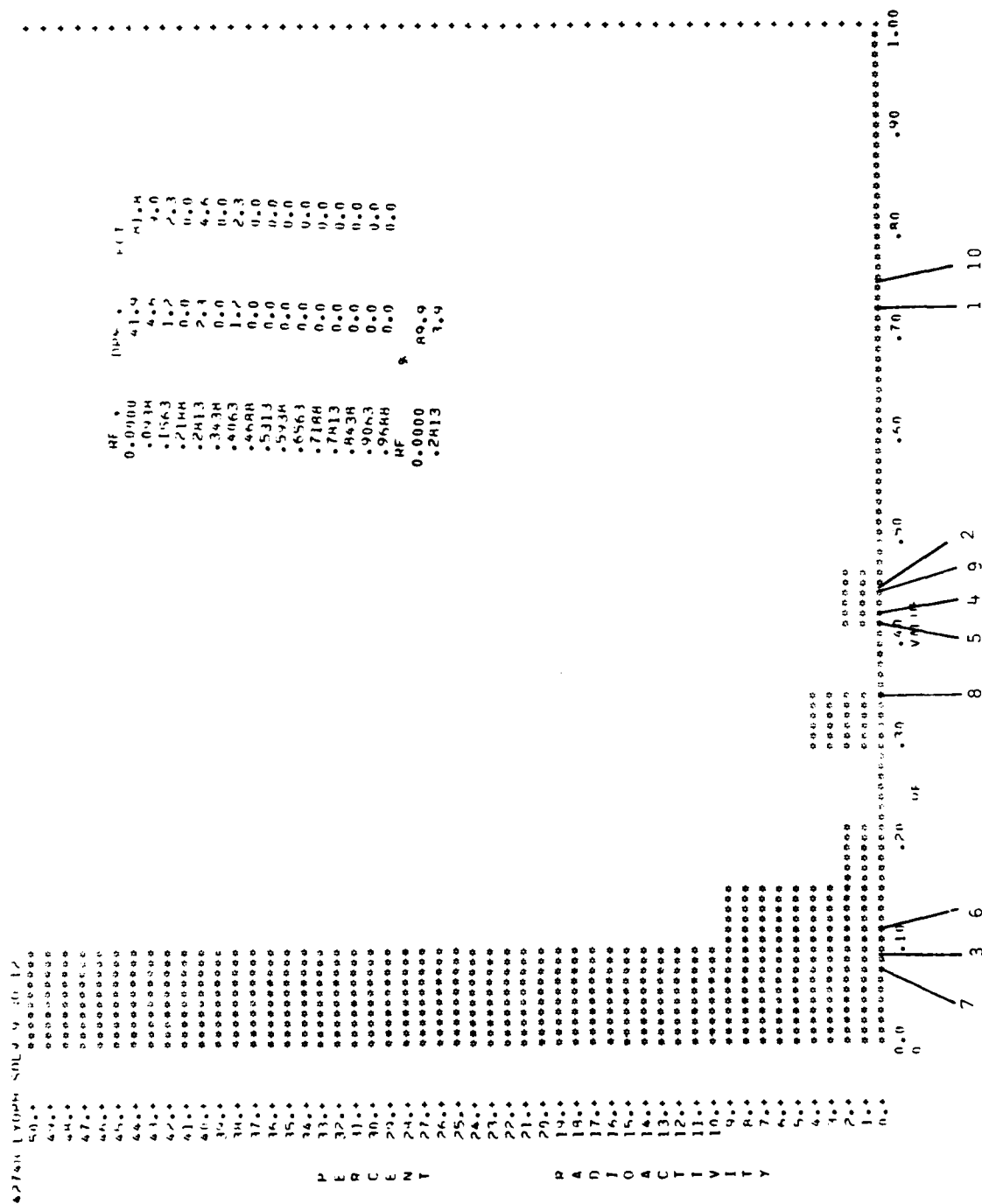


Figure 13-g-I: Male Rabbits, Oral Treatment, Solvent I



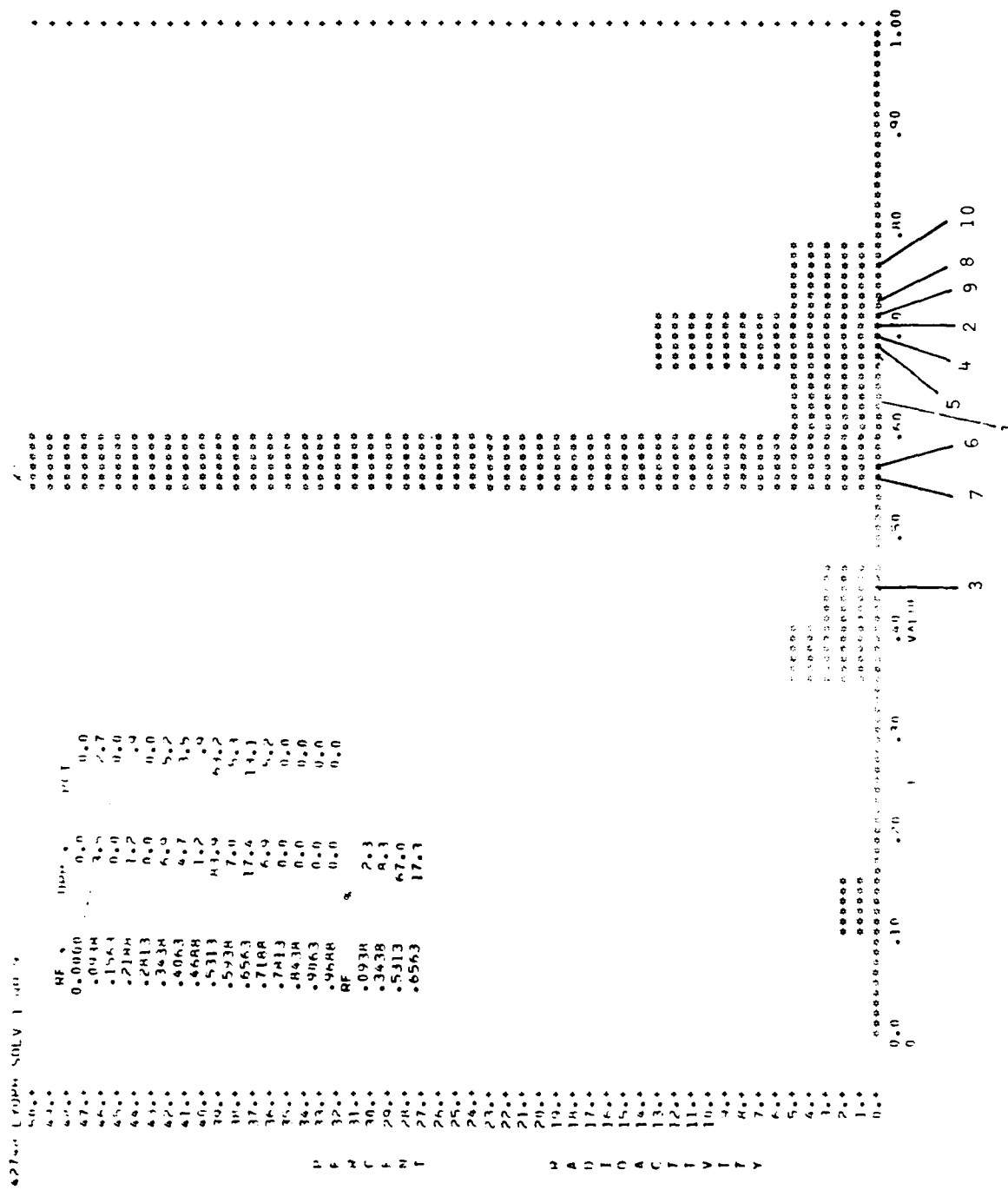


Figure 13-h-I: Male Rabbits, Dermal Application, Solvent I

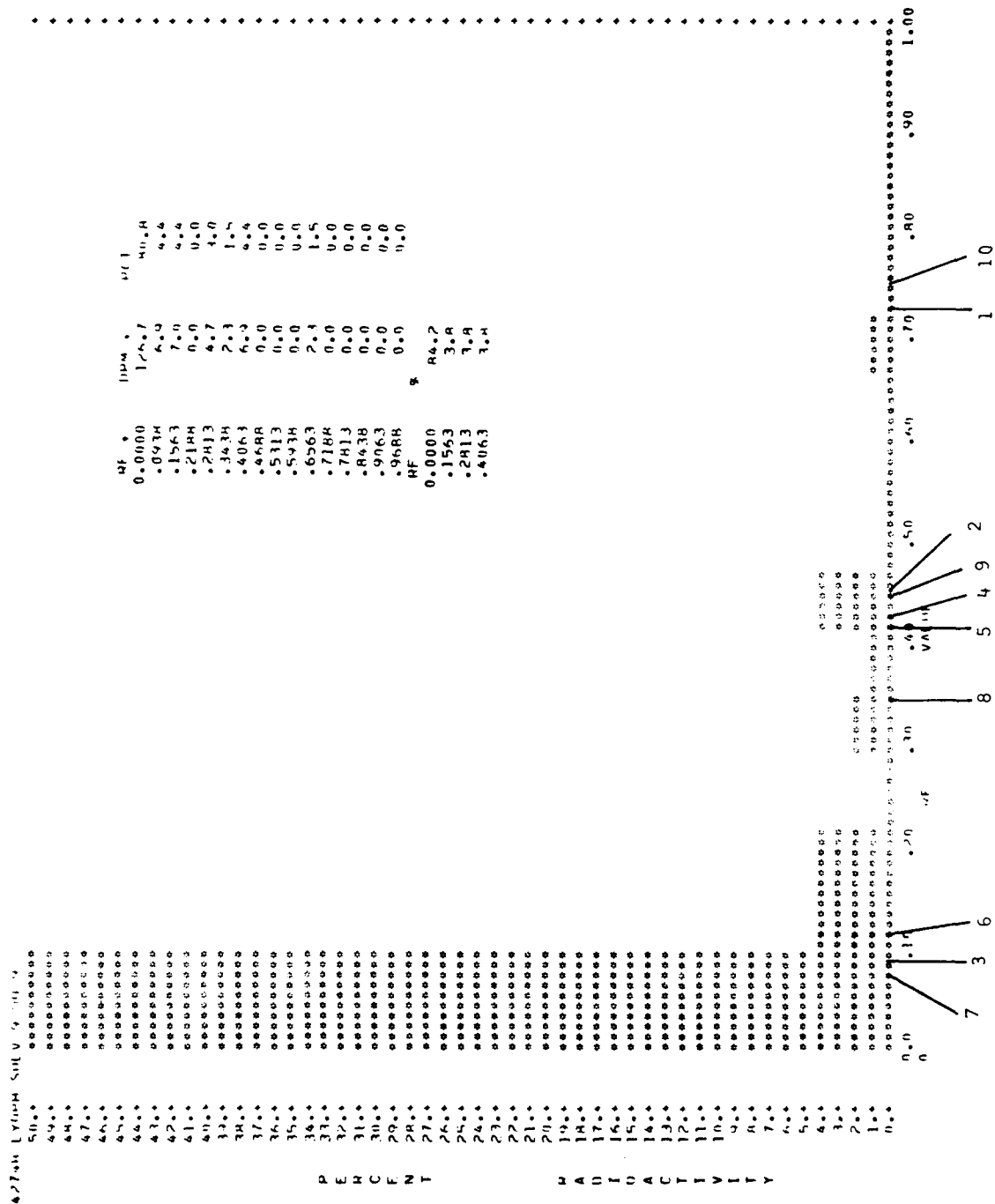


Figure 14: TLC of Lyophilized Urine Obtained from Rats, Mice, Rabbits and Dogs Treated Orally or Dermally with ^{14}C -TNT. Plates were cut into 0.5 cm zones. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 14 follows

42748 APRIL 28 1978 LYOPHYLIZED URINE .5 CM CUTS SOLVENT 1 NO 1

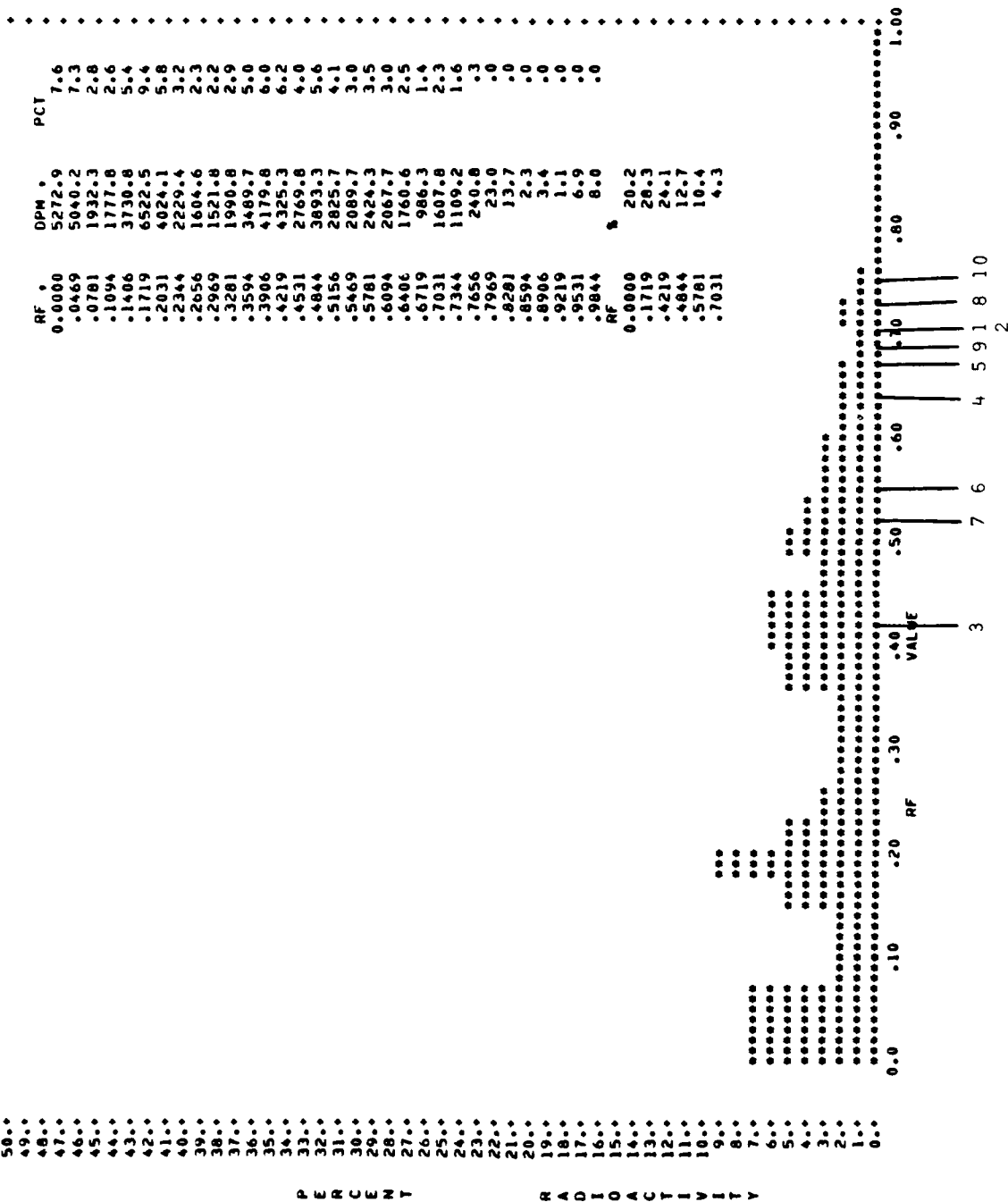


Figure 14-a-I: Male Rats, Oral Treatment, Solvent I

42748 APRIL 28 1978 LYOPHYLIZED URINE .5 CM CUTS SOLVENT 9 NO 1

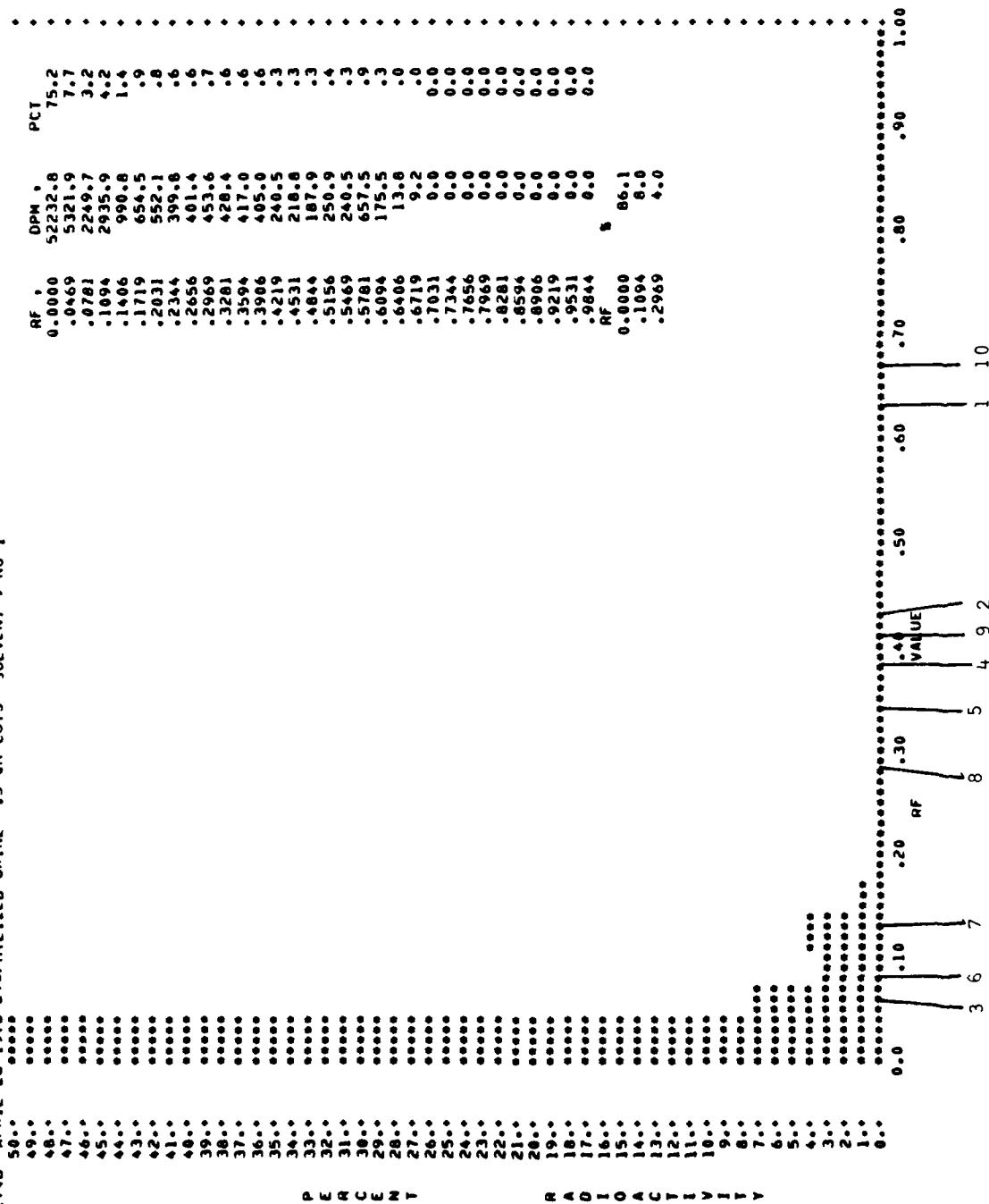


Figure 14-a-IX: Male Rats, Oral Treatment, Solvent IX

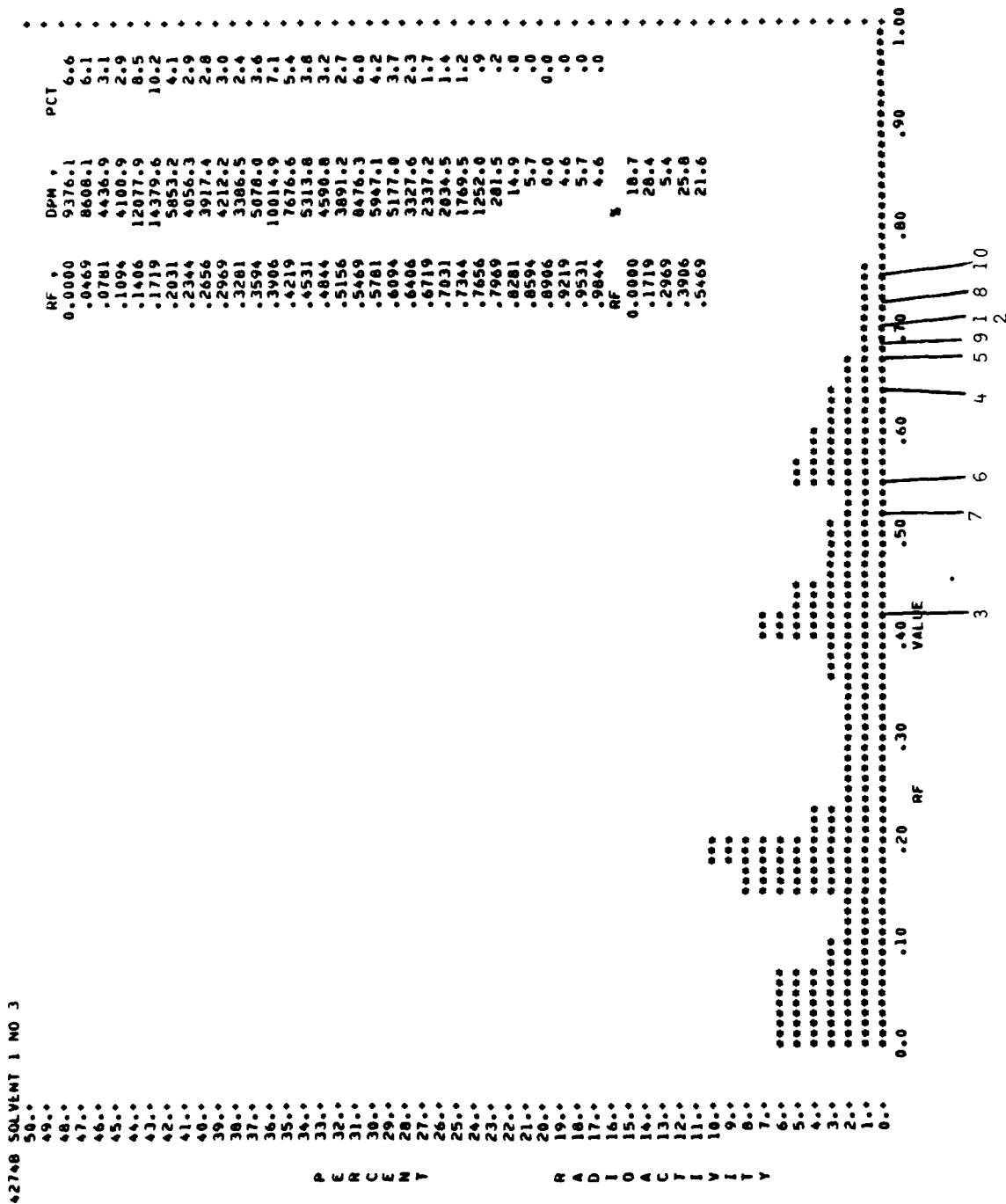


Figure 14-b-I: Female Rats, Oral Treatment, Solvent I

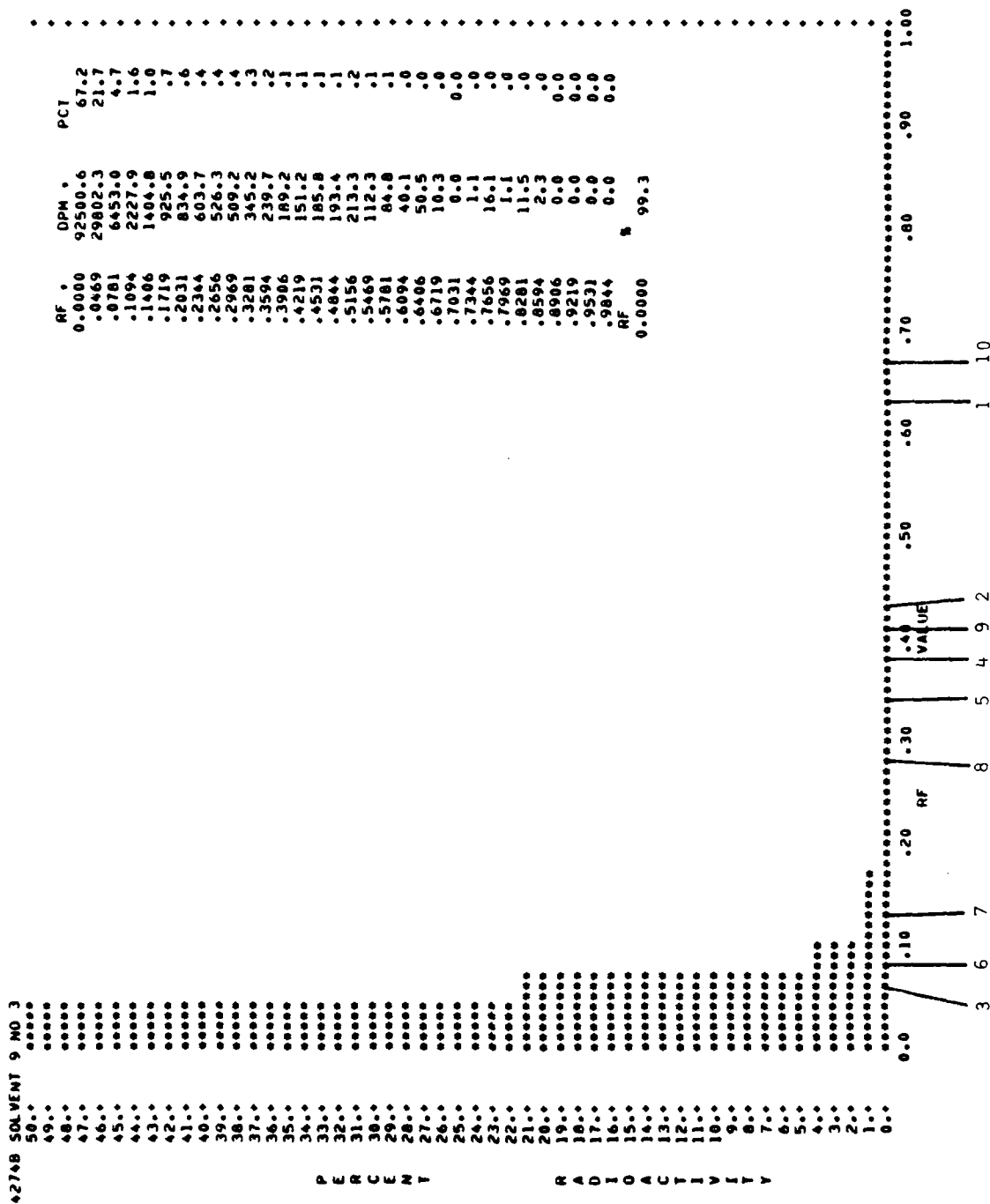


Figure 14-b-IX: Female Rats, Oral Treatment, Solvent IX

42748 SOLVENT 1 NO 4

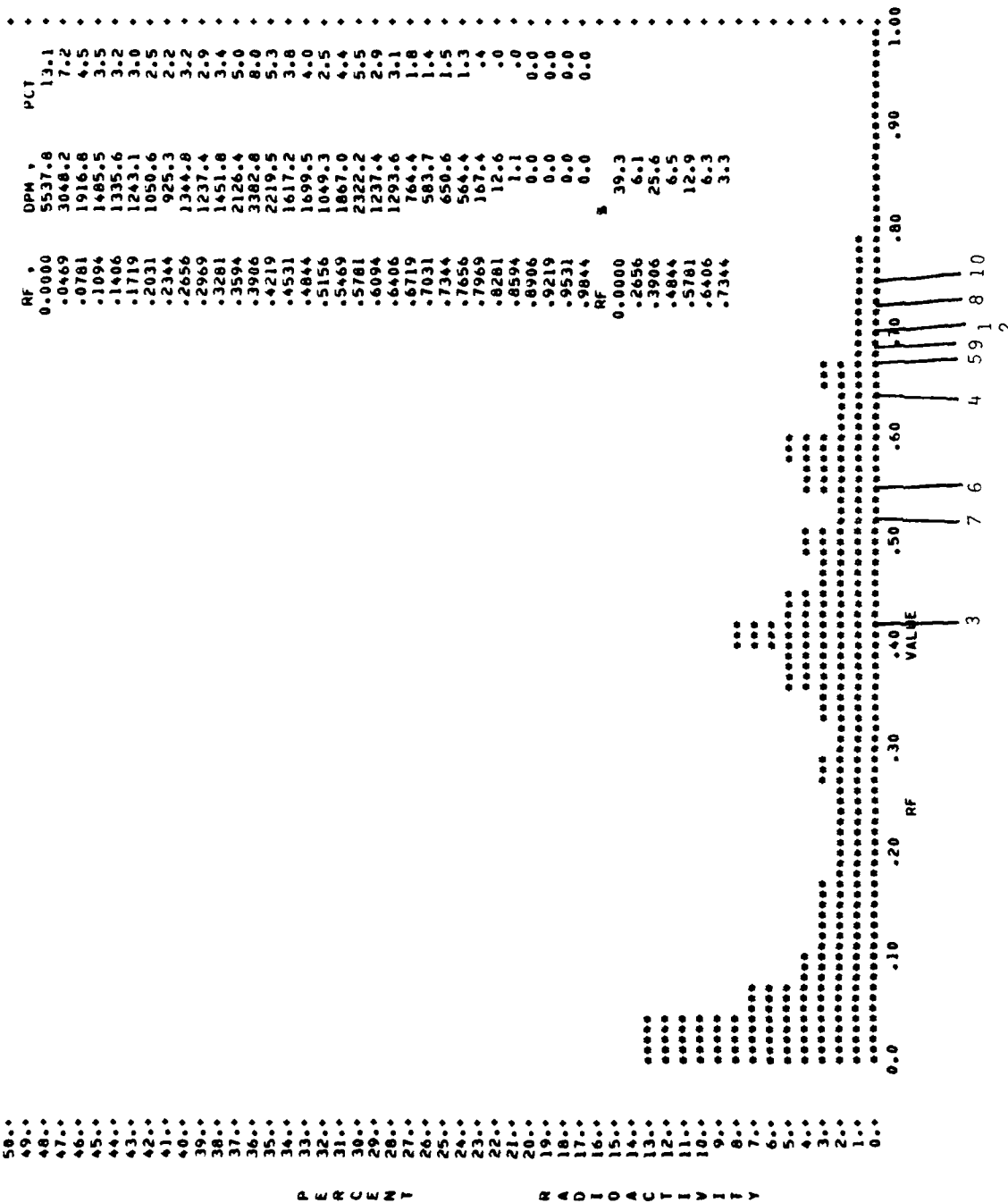


Figure 14-c-I: Male Rats, Dermal Application, Solvent I

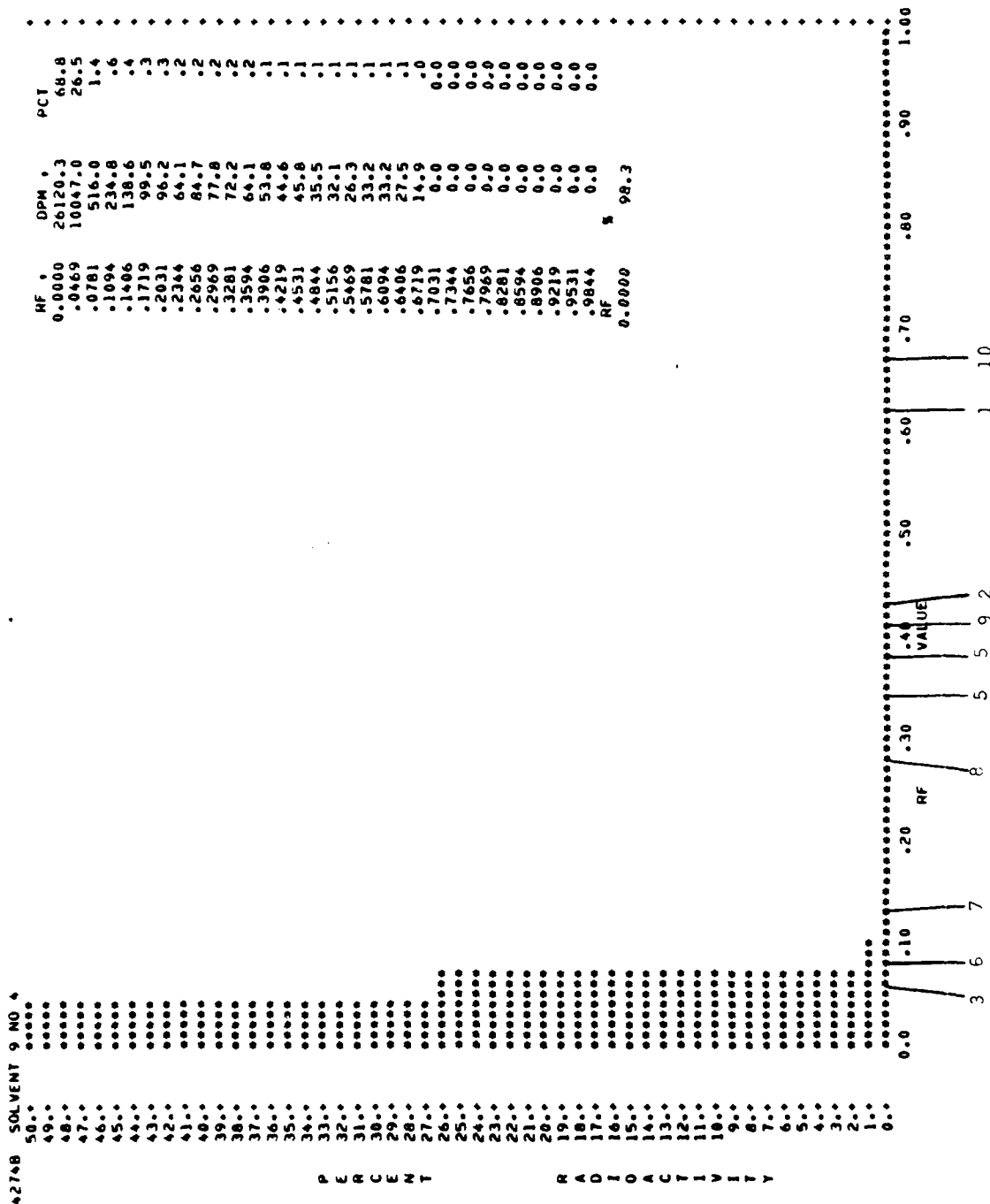


Figure 14-c-IX: Male Rats, Dermal Application, Solvent IX

4274B SOLVENT 1 NO 2

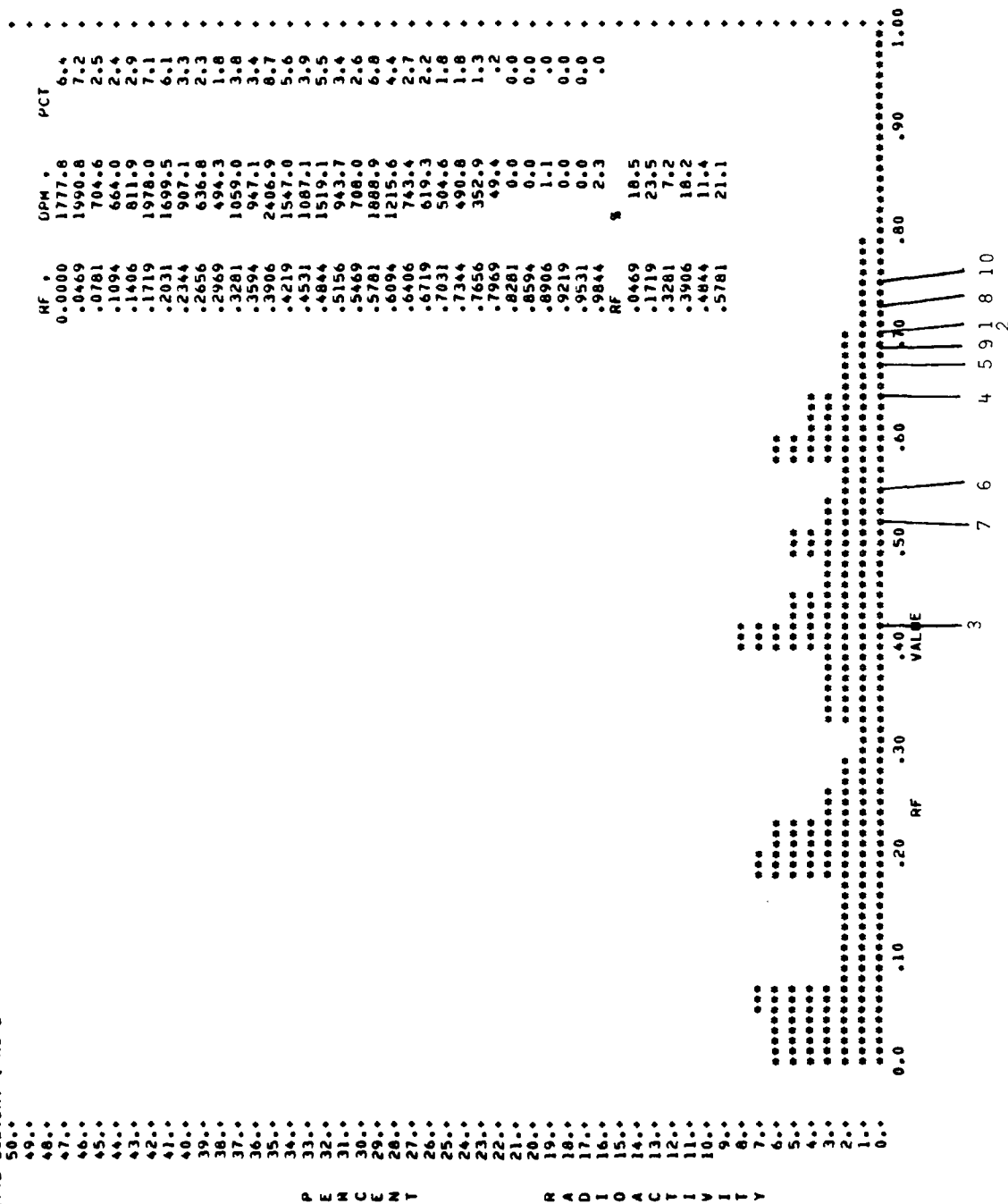


Figure 14-d-I: Female rats, Dermal Application, Solvent I

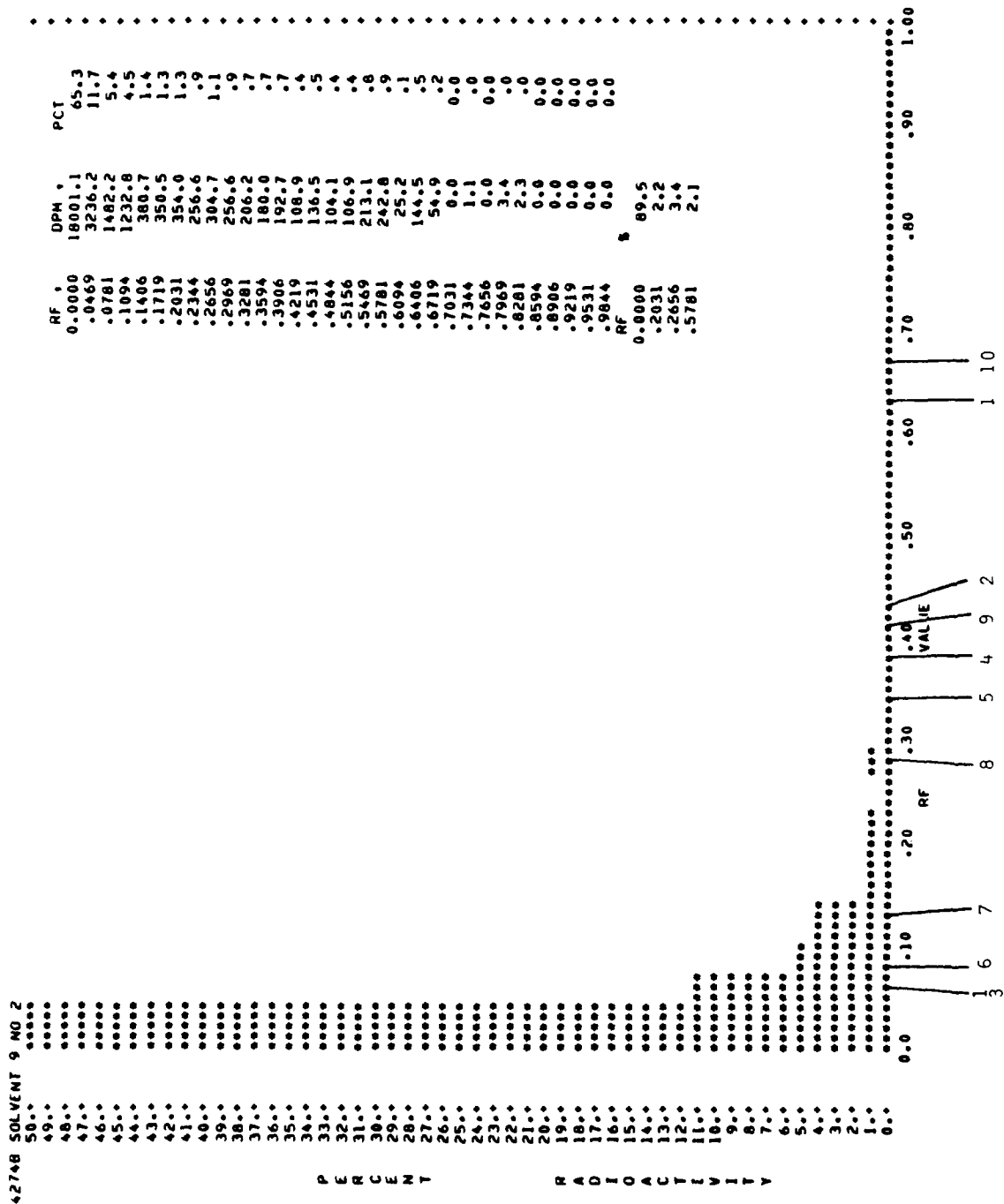


Figure 14-d-IX: Female Rats, Dermal Application, Solvent IX

RF	UPM	PCT
0.0000	2150.2	3.7
.0469	1813.3	3.1
.0781	1785.8	3.1
.1094	2980.5	5.1
.1406	2761.7	4.7
.1719	3662.1	6.3
.2031	5371.6	9.2
.2344	3824.5	6.6
.2656	2649.1	4.5
.2969	3037.8	5.2
.3281	1298.2	2.2
.3594	1034.5	1.8
.3906	1353.2	2.3
.4219	2609.4	4.5
.4531	2072.2	3.6
.4844	1788.1	3.1
.5156	2106.7	3.6
.5469	1863.7	3.2
.5781	2031.0	3.5
.6094	2010.3	3.5
.6406	1805.0	3.1
.6719	1714.9	2.9
.7031	1918.6	3.3
.7344	2722.5	4.7
.7656	1341.7	2.3
.7969	447.2	.8
.8281	44.7	.1
.8594	32.1	.1
.8906	14.9	.0
.9219	9.2	.0
.9531	10.3	.0
.9844	2.3	.0
RF		
0.0000	9.9	
.1094	9.9	
.2031	26.6	
.2969	9.2	
.4219	13.4	
.5156	6.8	
.5781	13.0	
.7344	11.2	

RF

VALVE

3 4 5 6 7 8 9 10

Figure 14-e-1: Male Mice, Oral Treatment, Solvent I

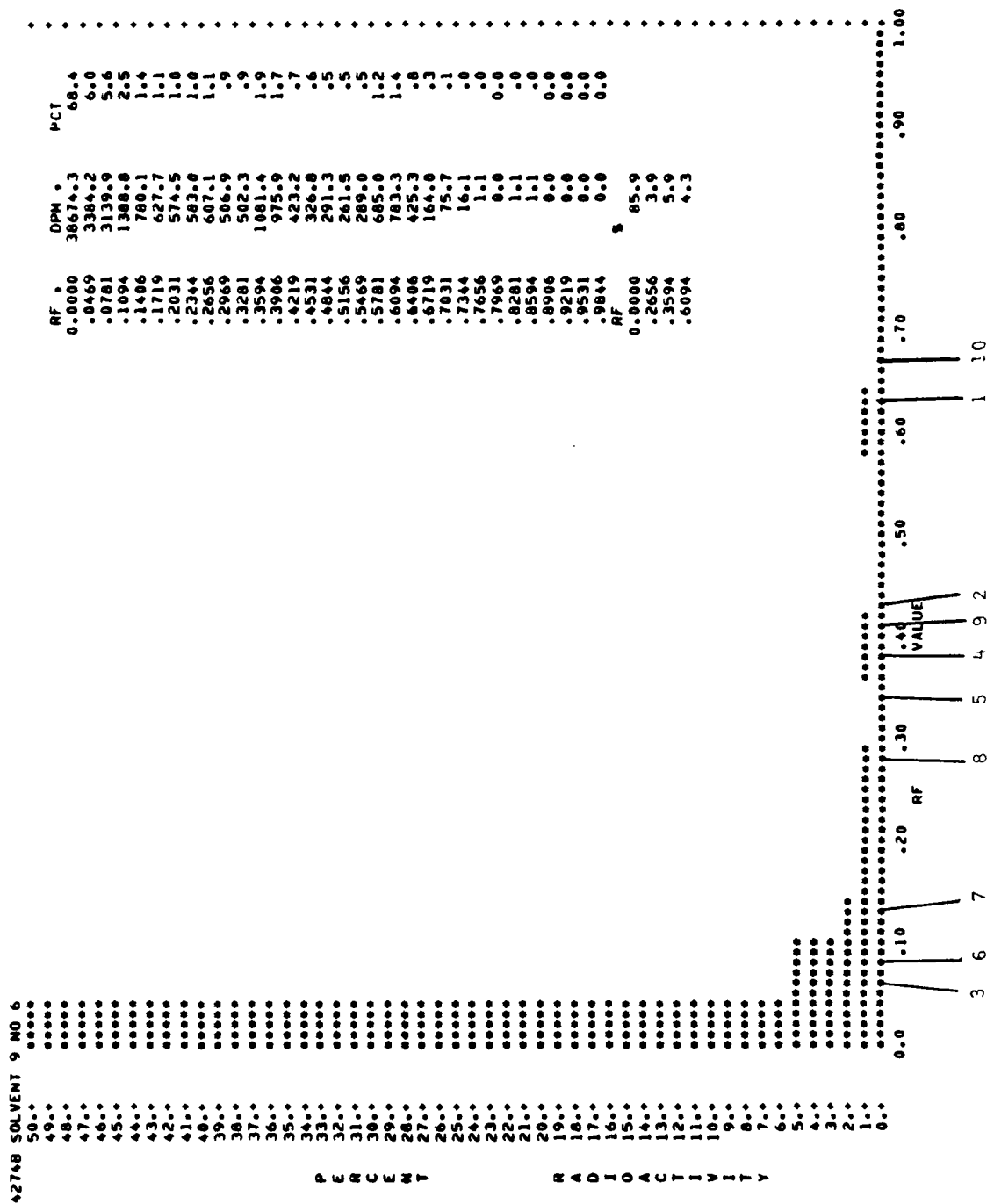


Figure 14-e-IX: Male Mice, Oral Treatment, Solvent IX

42748 SOLVENT 1 NO 5

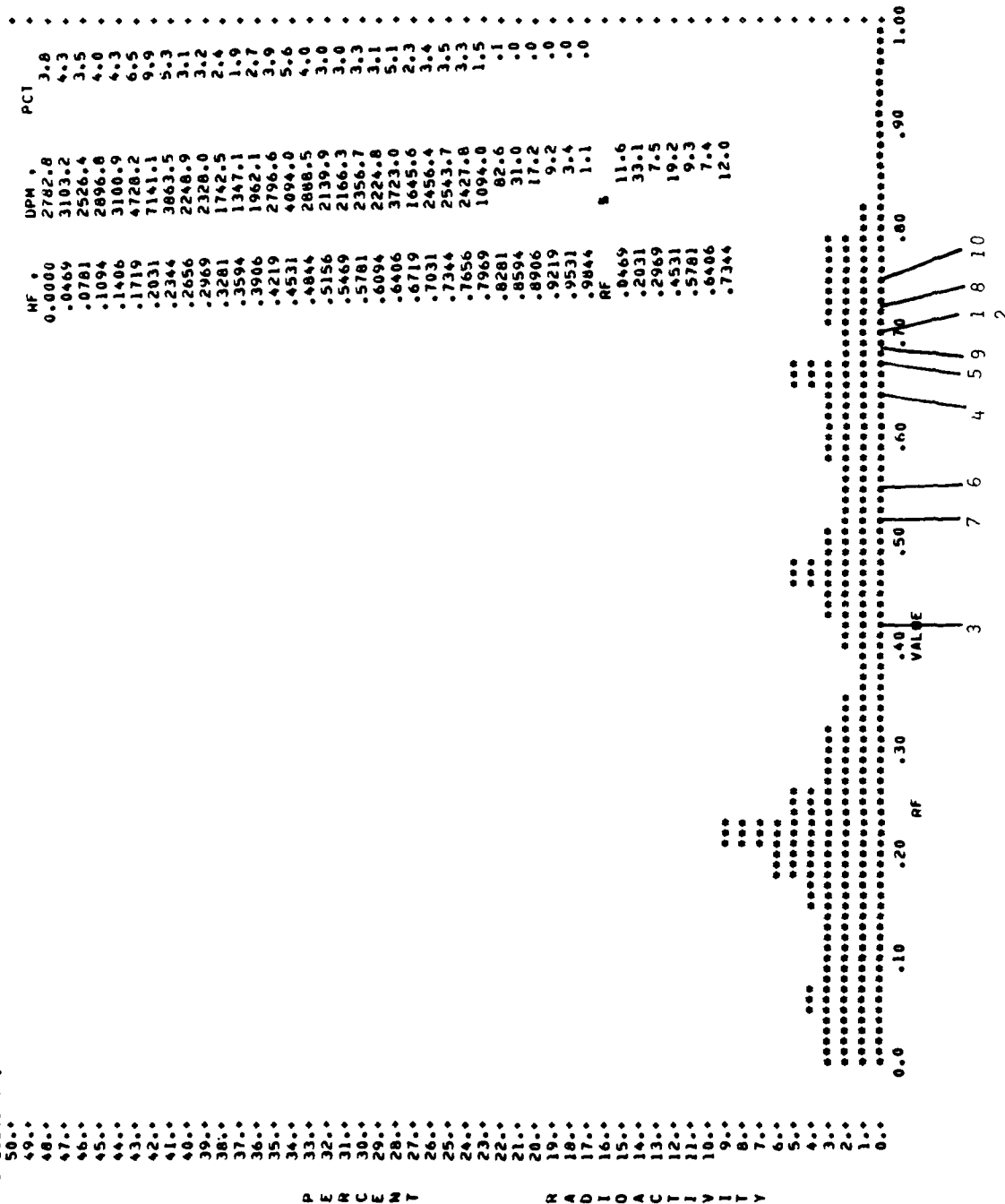


Figure 14-f-I: Male Mice, Dermal Application, Solvent I

	RF ,	DPM ,	PCT
0.0000	45244.0	65.7	
.0469	4308.5	6.3	
.0781	4342.5	6.3	
.1094	2397.9	3.5	
.1406	971.4	1.4	
.1719	608.7	1.2	
.2031	682.7	1.0	
.2344	675.5	1.0	
.2656	666.3	1.0	
.2969	835.1	1.2	
.3281	735.7	1.1	
.3594	1256.9	1.0	
.3906	1056.1	1.5	
.4219	664.0	1.0	
.4531	559.8	.8	
.4844	462.8	.7	
.5156	450.2	.7	
.5469	450.7	.7	
.5781	691.1	1.0	
.6094	673.5	1.0	
.6406	335.6	.5	
.6719	431.2	.6	
.7031	101.2	.3	
.7344	14.9	.0	
.7656	0.0	0.0	
.7969	0.0	0.0	
.8281	0.0	0.0	
.8594	2.3	.0	
.8906	0.0	0.0	
.9219	0.0	0.0	
.9531	1.1	.0	
.9844	0.0	0.0	
RF			
0.0000	71.9		
.0781	15.3		
.2969	2.3		
.3594	7.1		
.5781	2.5		

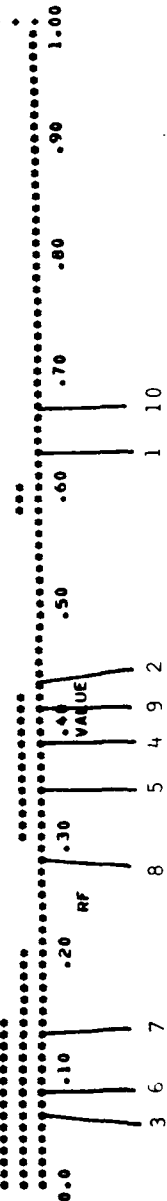


Figure 14-f-IX: Male Mice, Dermal Application, Solvent IX

42746 SOLVENT 1 NO 8

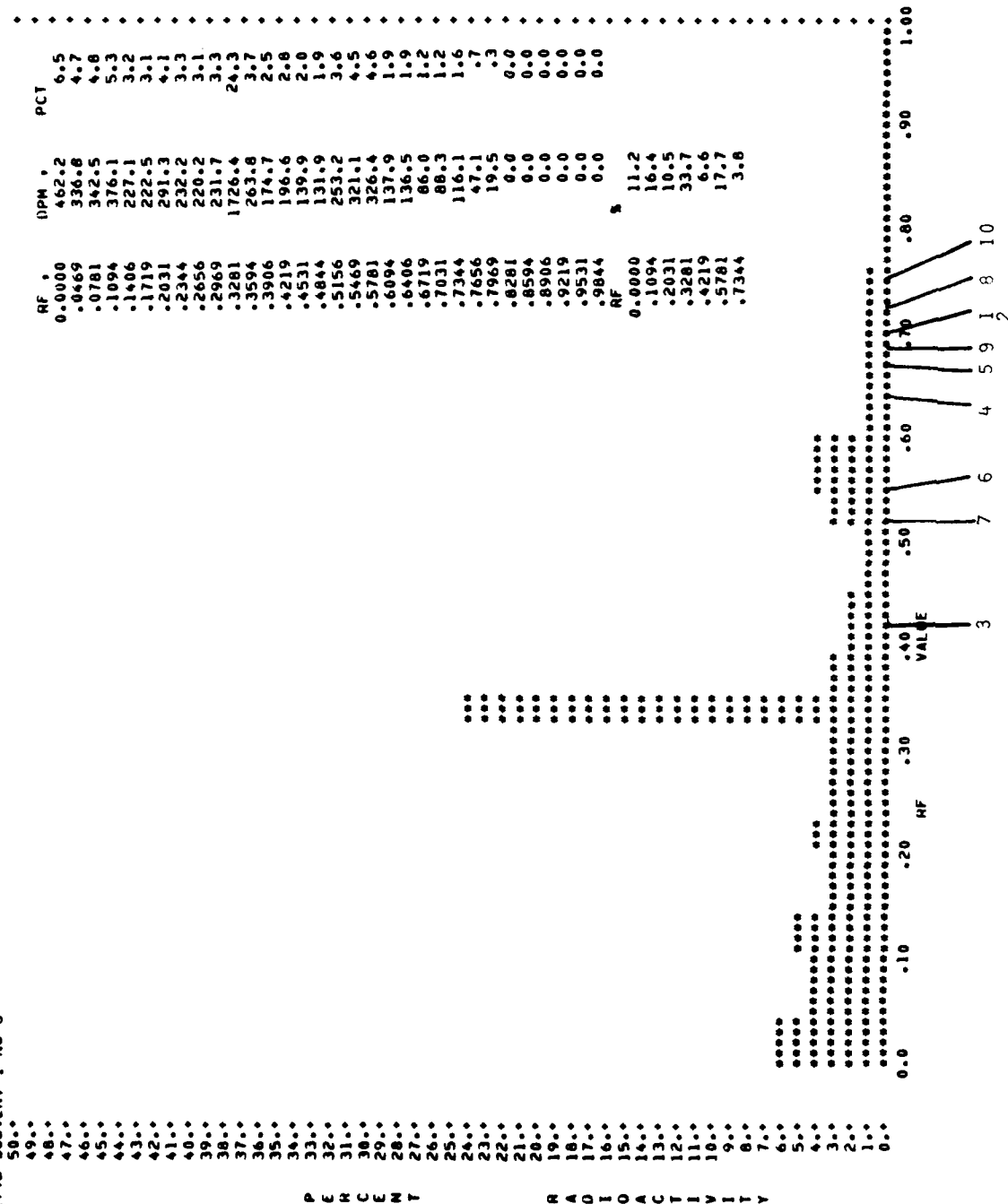


Figure 14-g-I: Male Rabbits, Oral Treatment, Solvent I

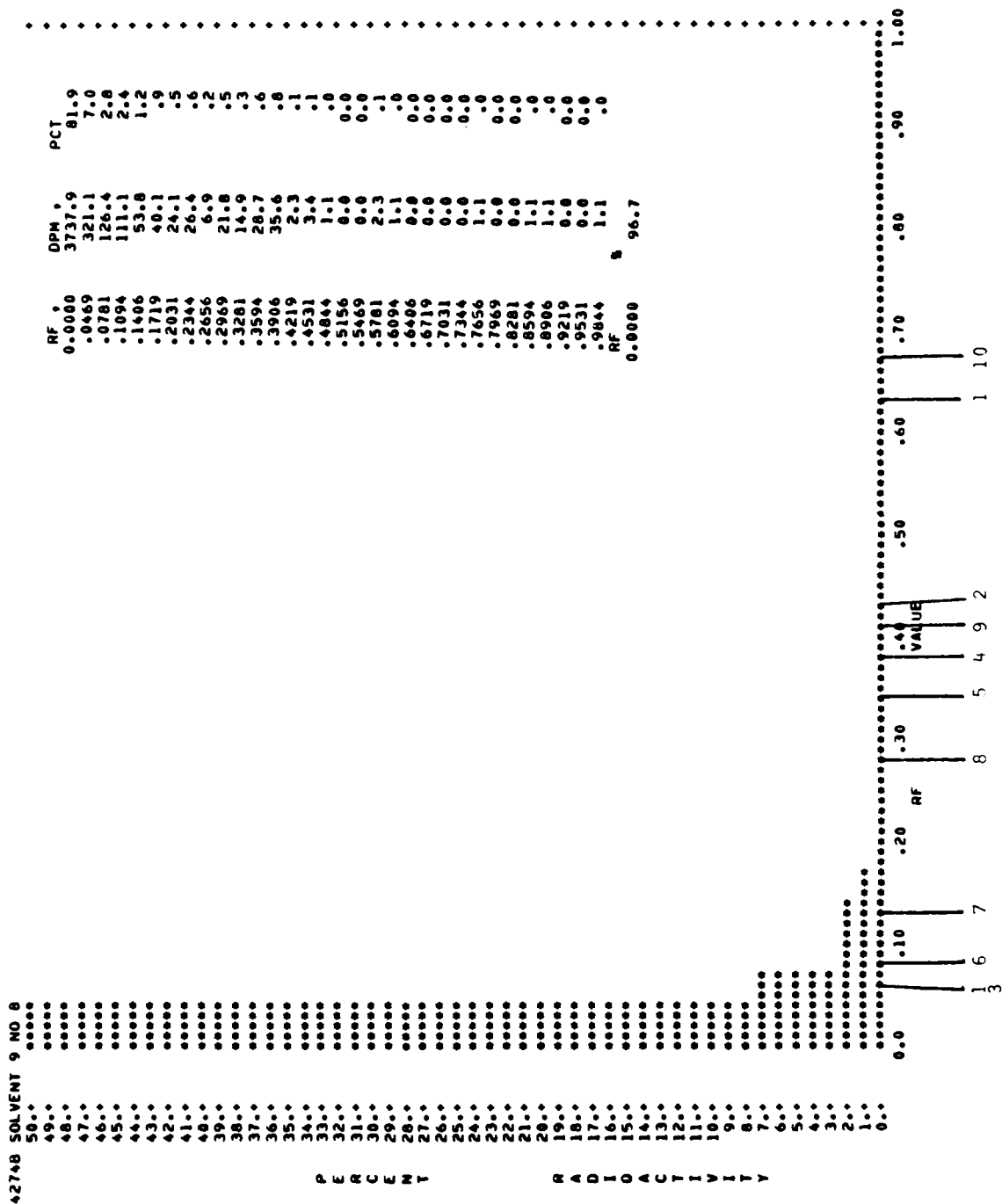


Figure 14-g-IX: Male Rabbits, Oral Treatment, Solvent IX

4274B SOLVENT 1 NO 7

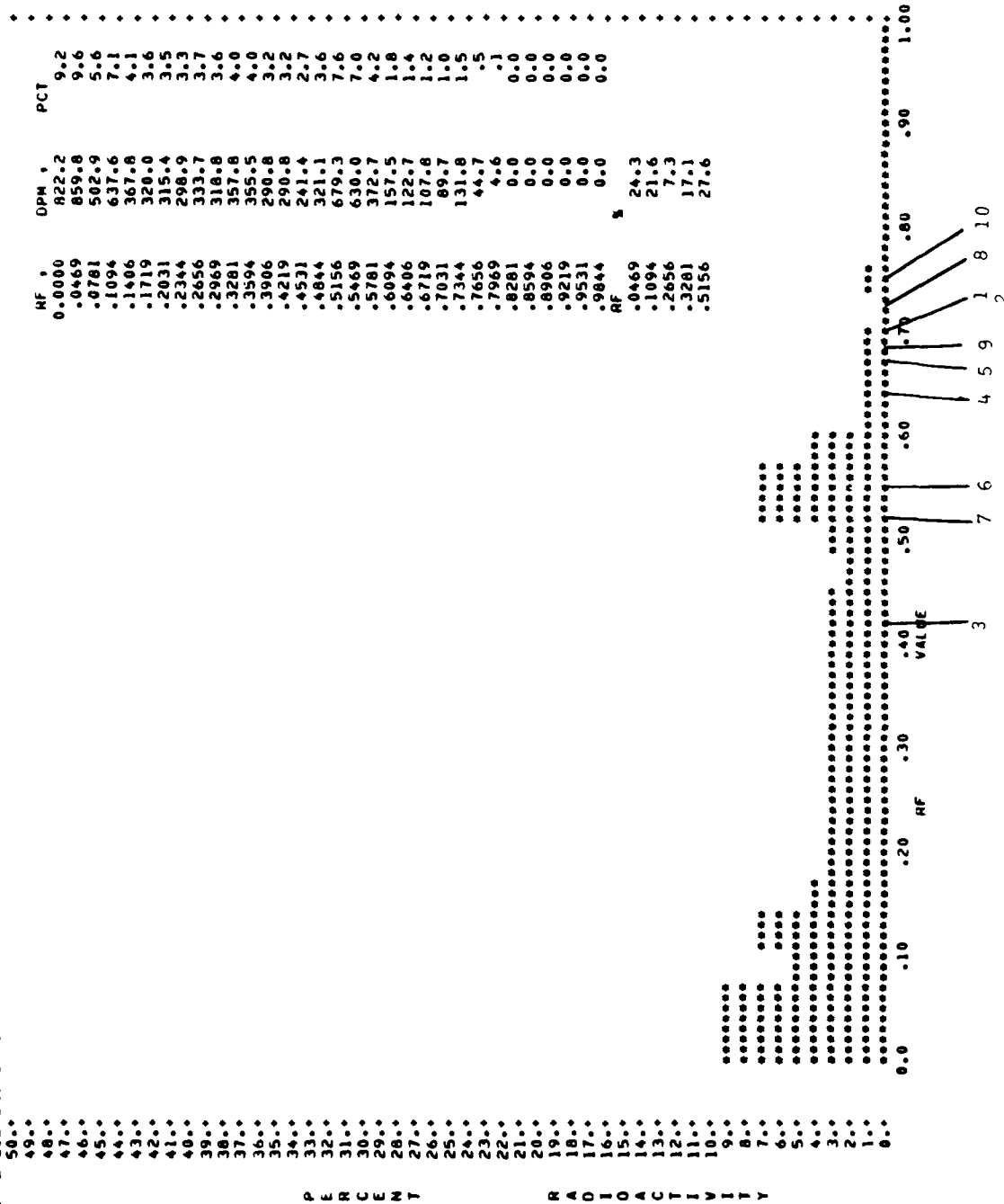


Figure 14-h-I: Male Rabbits, Dermal Application, Solvent I

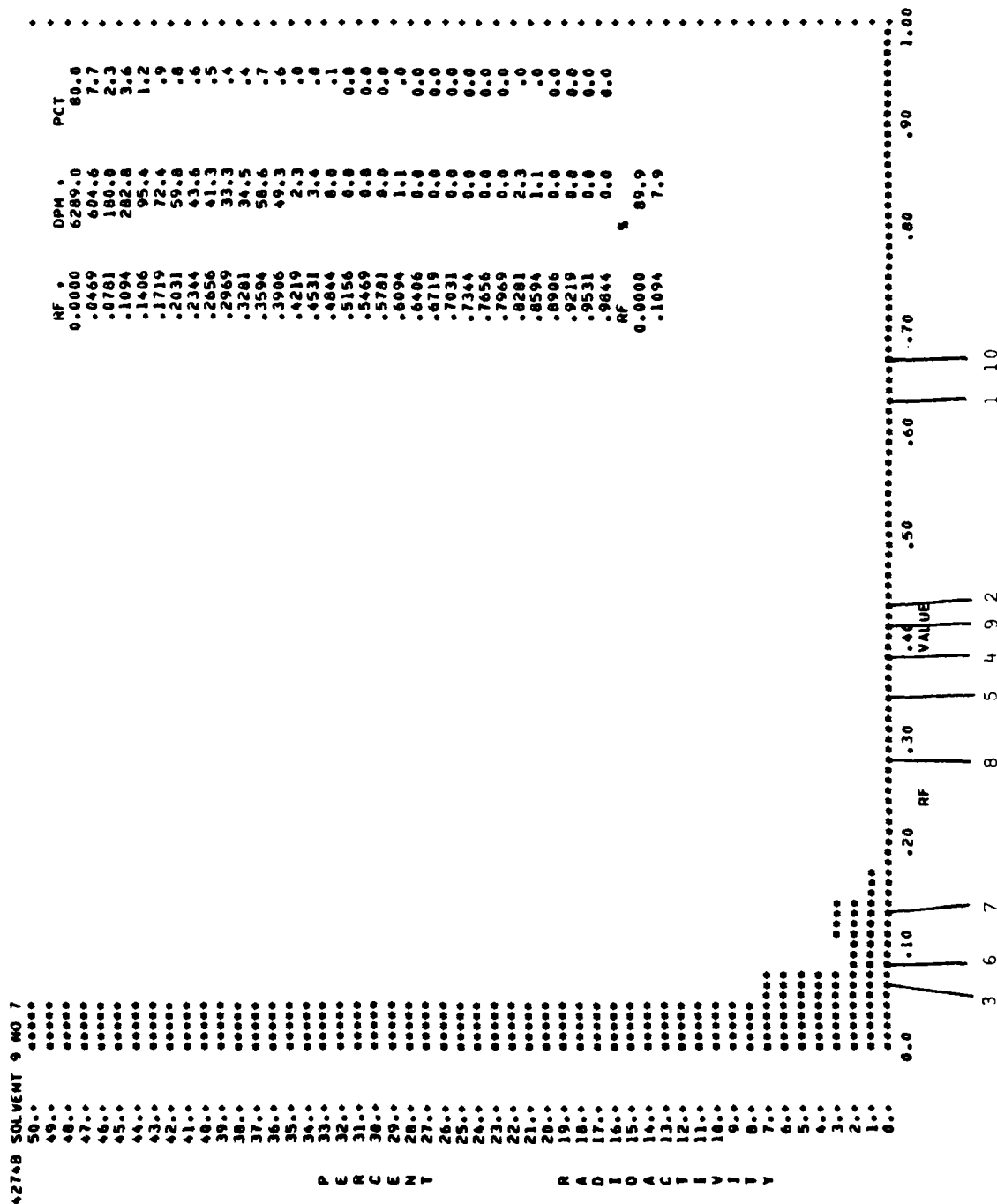


Figure 14-h-IX: Male Rabbits, Dermal Application, Solvent IX

42748 SOLVENT 1 NO 10

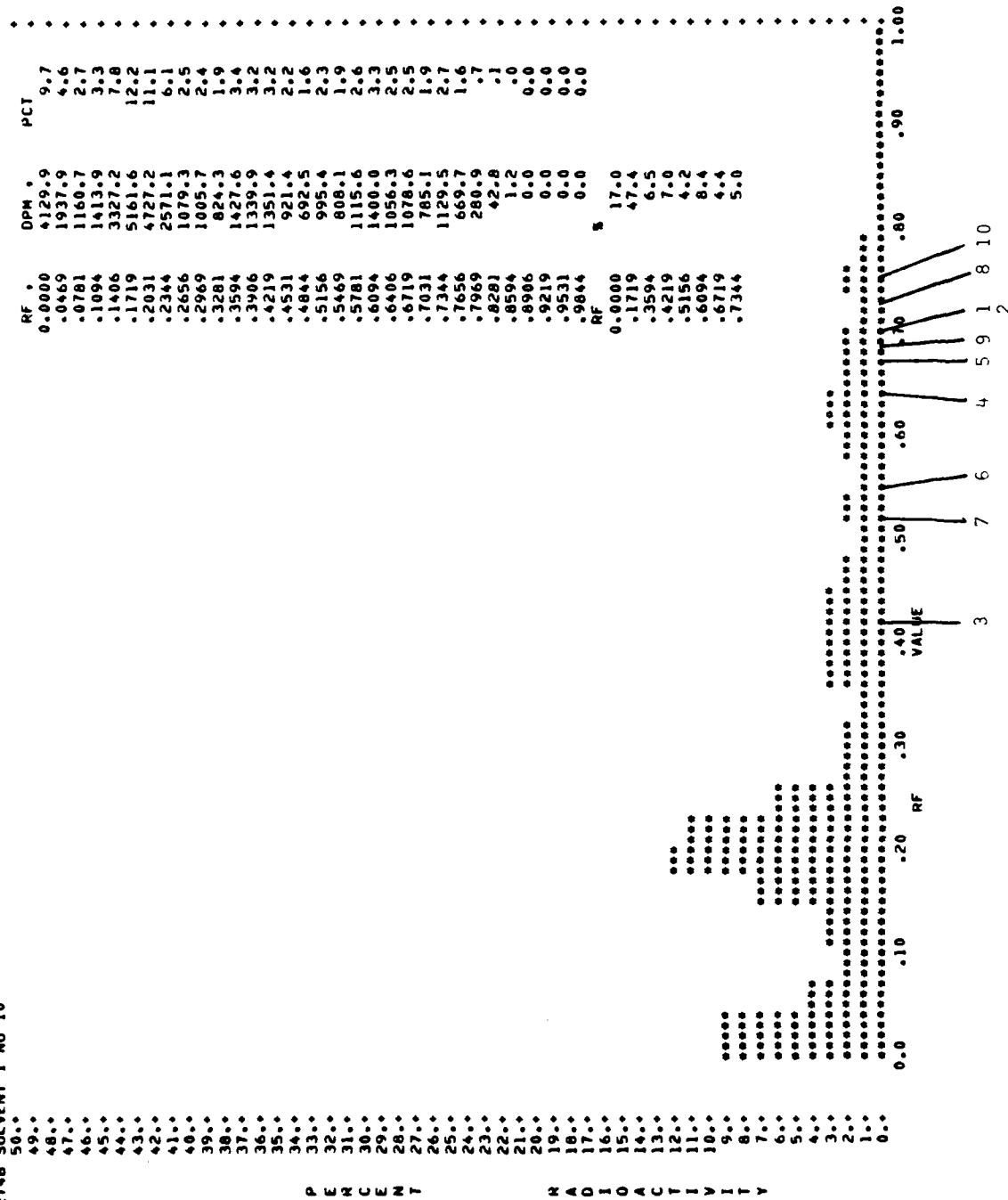


Figure 14-k-I: Male Dogs, Oral Treatment, Solvent I



4274B SOLVENT 1 NO 9

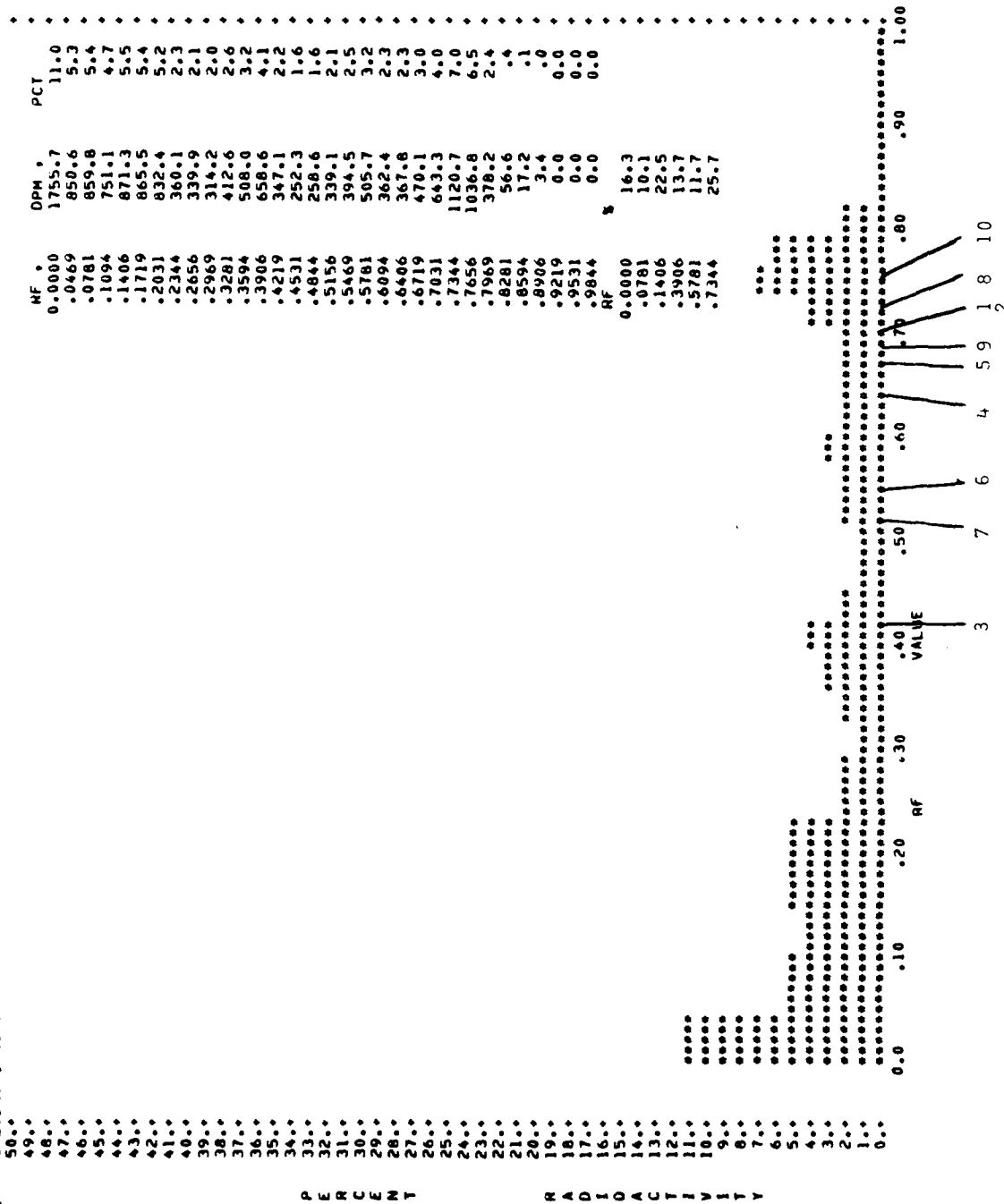


Figure 14-1-I: Male Dogs, Dermal Application, Solvent I

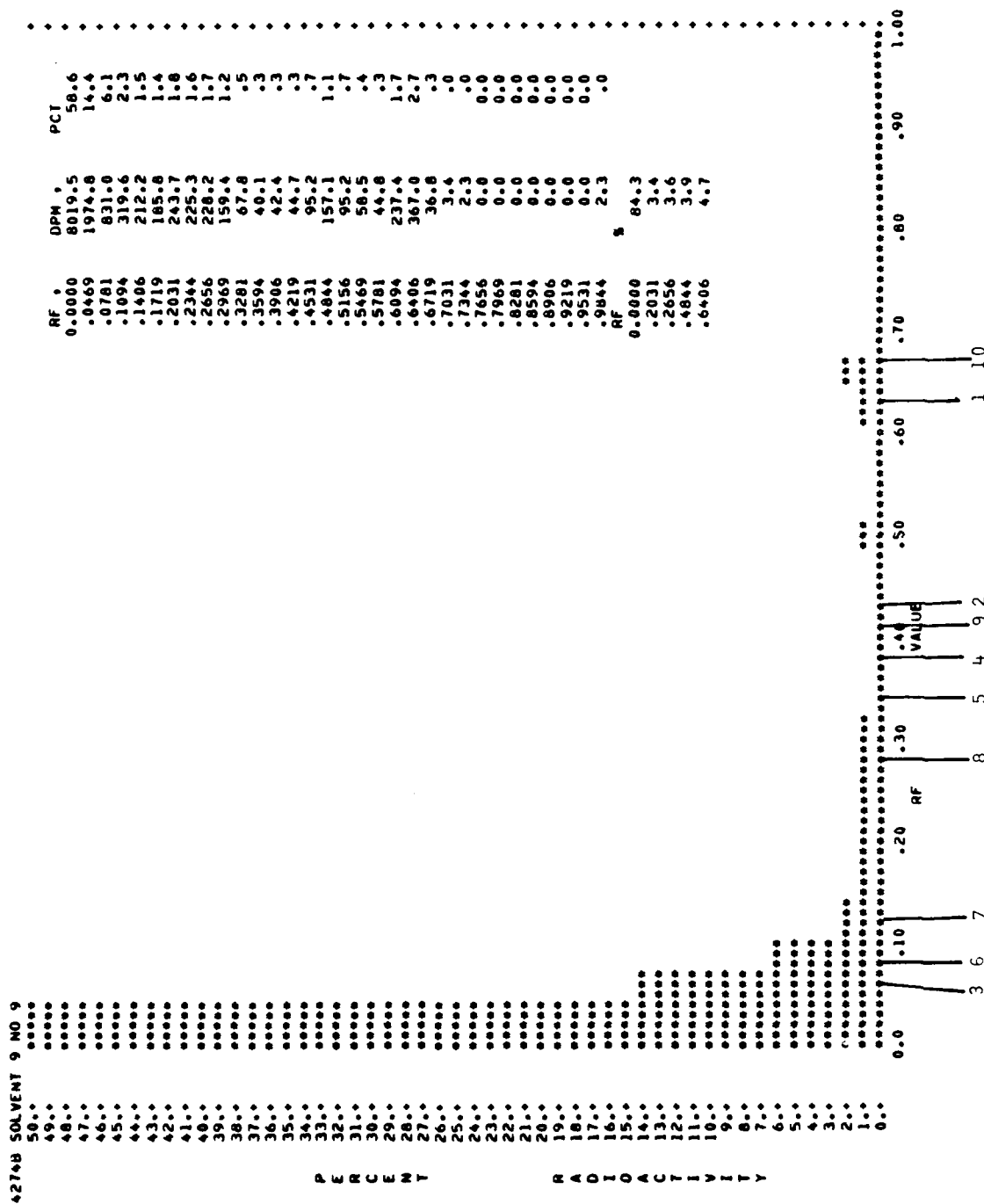


Figure 14-1-IX: Male Dogs, Dermal Application, Solvent IX

Figure 15: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Rats Treated Orally with ^{14}C -TNT. Prior to extraction, urine samples were incubated with acetate buffer and β -glucuronidase or aryl-sulfatase control samples were incubated with acetate buffer and water. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 15 follows

4274M SOLVENT 1 NO 1

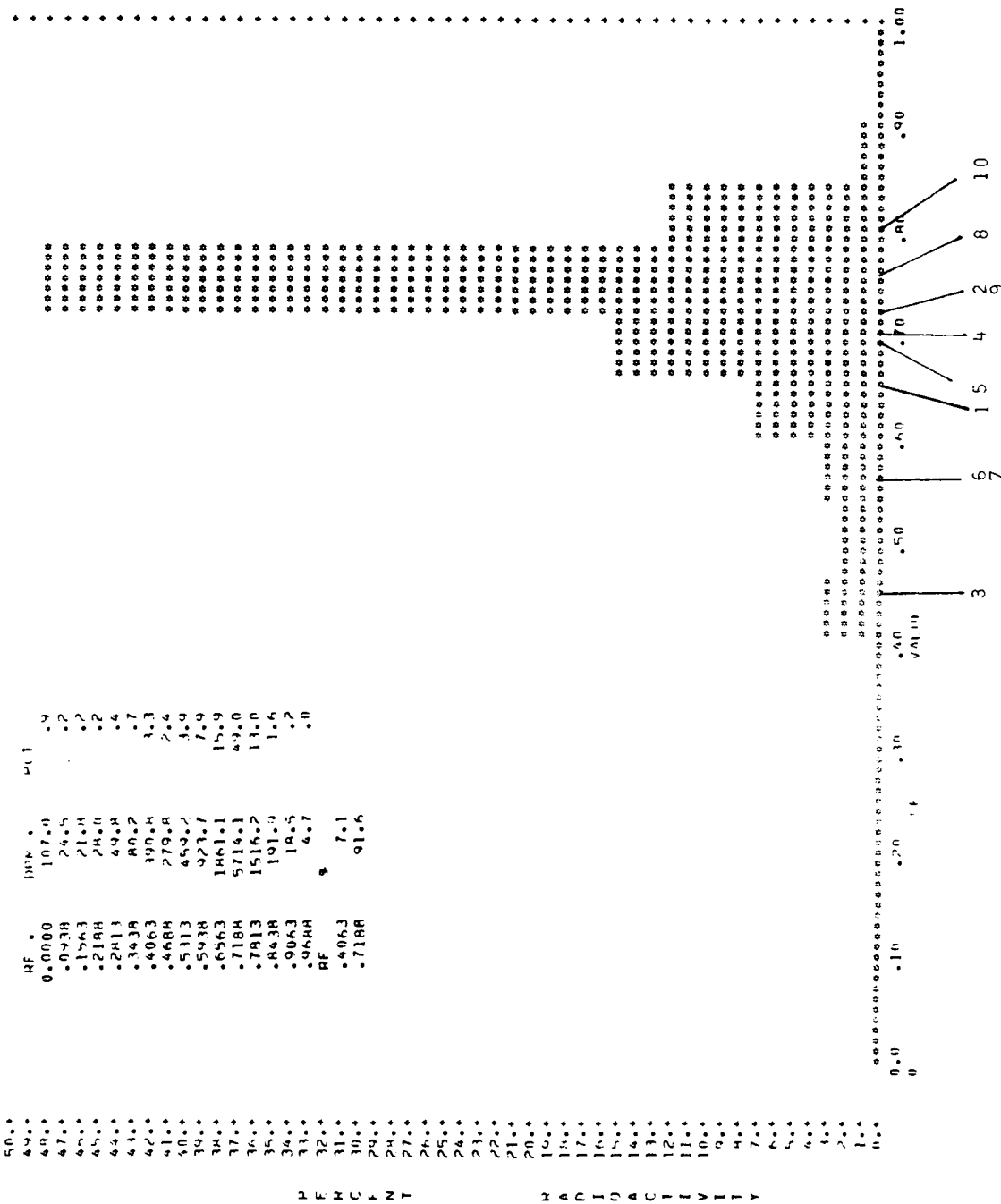


Figure 15-a-I: Male Rats, Incubation with Water, Solvent I

42746 SOLV 9 90 1 Exp (6000 26 1977

P	50.0	RF	10.4	1.1
E	4.0	0.0000	14.4	1.2
W	4.0	.0378	14.4	1.2
C	4.0	.1563	10.4	1.2
F	4.0	.2184	7.4	1.2
N	4.0	.2413	14.4	1.2
T	4.0	.3438	14.4	1.2
	4.0	.4063	14.4	1.2
	4.0	.4684	14.4	1.2
	4.0	.5313	14.4	1.2
	4.0	.5938	14.4	1.2
	4.0	.6563	14.4	1.2
	4.0	.7184	14.4	1.2
	4.0	.7813	14.4	1.2
	4.0	.8438	14.4	1.2
	4.0	.9063	14.4	1.2
	4.0	.9684	14.4	1.2
	4.0	RF	10.4	1.1
	4.0	0.0000	14.4	1.2
	4.0	.3438	14.4	1.2
	4.0	.6563	14.4	1.2

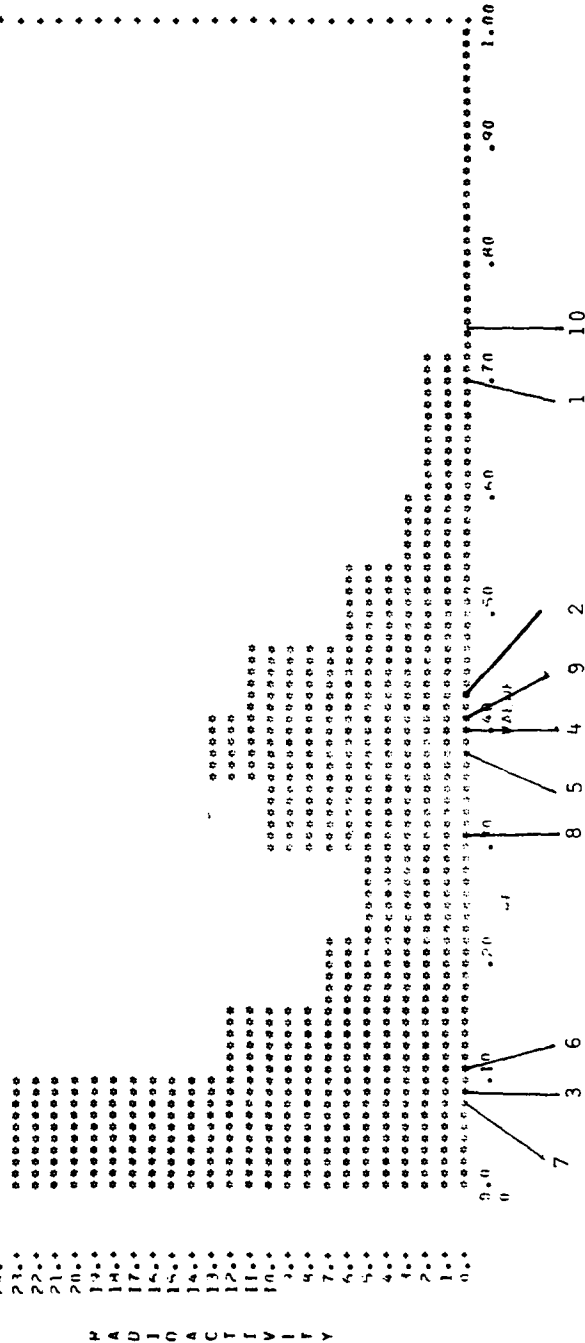


Figure 15-a-IX: Male Rats, Incubation with Water, Solvent IX

4.274H SOLVENT I 100.0

50.0	0.0000	100.0	0.0
49.0	0.0000	100.0	0.0
48.0	0.0000	100.0	0.0
47.0	0.0000	100.0	0.0
46.0	0.0000	100.0	0.0
45.0	0.0000	100.0	0.0
44.0	0.0000	100.0	0.0
43.0	0.0000	100.0	0.0
42.0	0.0000	100.0	0.0
41.0	0.0000	100.0	0.0
40.0	0.0000	100.0	0.0
39.0	0.0000	100.0	0.0
38.0	0.0000	100.0	0.0
37.0	0.0000	100.0	0.0
36.0	0.0000	100.0	0.0
35.0	0.0000	100.0	0.0
34.0	0.0000	100.0	0.0
33.0	0.0000	100.0	0.0
32.0	0.0000	100.0	0.0
31.0	0.0000	100.0	0.0
30.0	0.0000	100.0	0.0
29.0	0.0000	100.0	0.0
28.0	0.0000	100.0	0.0
27.0	0.0000	100.0	0.0
26.0	0.0000	100.0	0.0
25.0	0.0000	100.0	0.0
24.0	0.0000	100.0	0.0
23.0	0.0000	100.0	0.0
22.0	0.0000	100.0	0.0
21.0	0.0000	100.0	0.0
20.0	0.0000	100.0	0.0
19.0	0.0000	100.0	0.0
18.0	0.0000	100.0	0.0
17.0	0.0000	100.0	0.0
16.0	0.0000	100.0	0.0
15.0	0.0000	100.0	0.0
14.0	0.0000	100.0	0.0
13.0	0.0000	100.0	0.0
12.0	0.0000	100.0	0.0
11.0	0.0000	100.0	0.0
10.0	0.0000	100.0	0.0
9.0	0.0000	100.0	0.0
8.0	0.0000	100.0	0.0
7.0	0.0000	100.0	0.0
6.0	0.0000	100.0	0.0
5.0	0.0000	100.0	0.0
4.0	0.0000	100.0	0.0
3.0	0.0000	100.0	0.0
2.0	0.0000	100.0	0.0
1.0	0.0000	100.0	0.0
0.0	0.0000	100.0	0.0

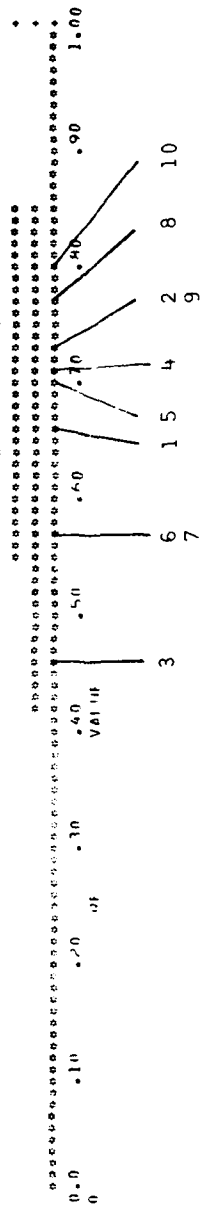


Figure 15-b-I: Male Rats, Incubation with β -Glucuronidase, Solvent I

4274R SOLV 4 NO 2 F 40 NOV 24 1977

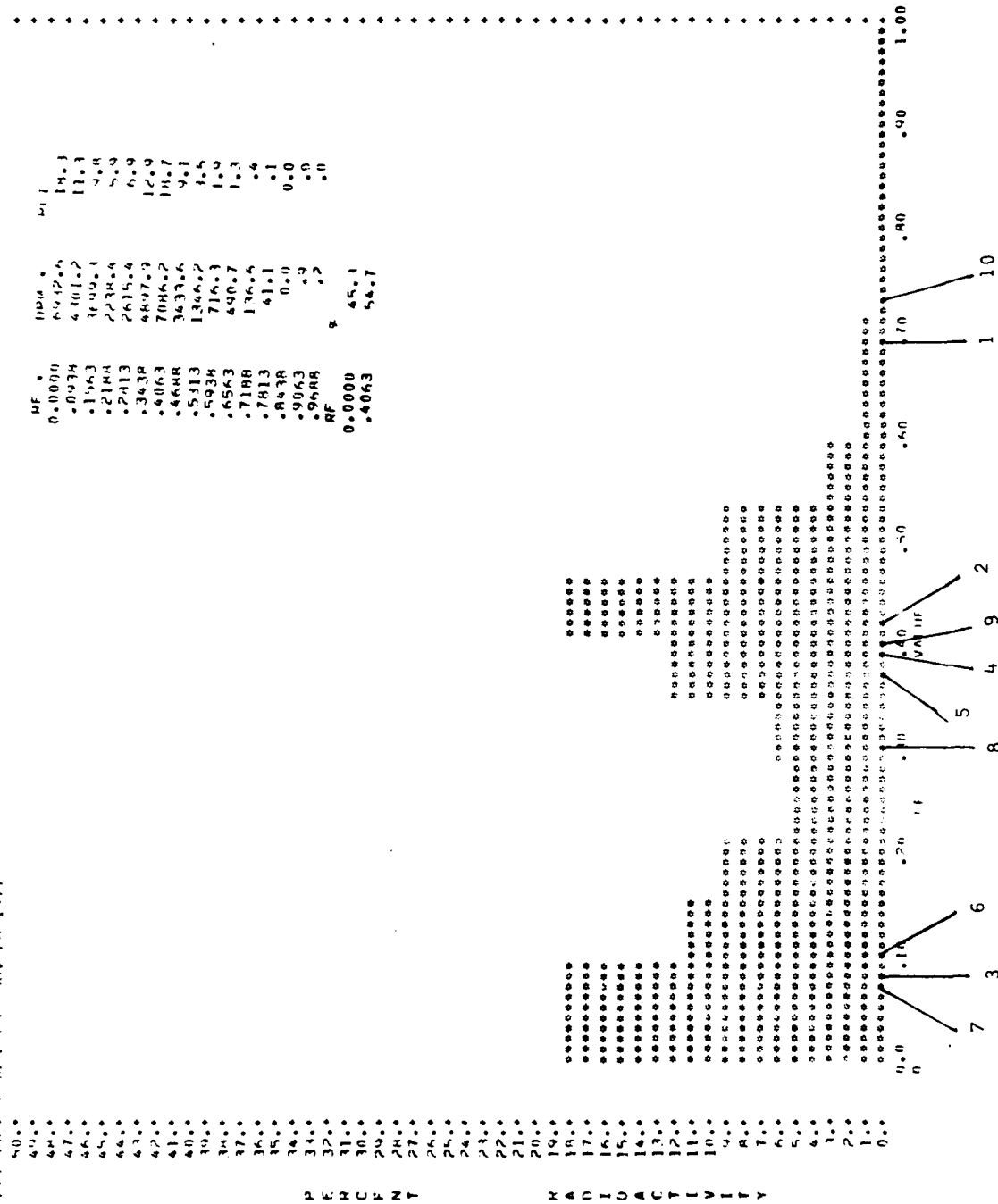


Figure 15-b-IX: Male Rats, Incubation with β -Glucuronidase, Solvent IX

42744 SOLVENT 1 NO 4

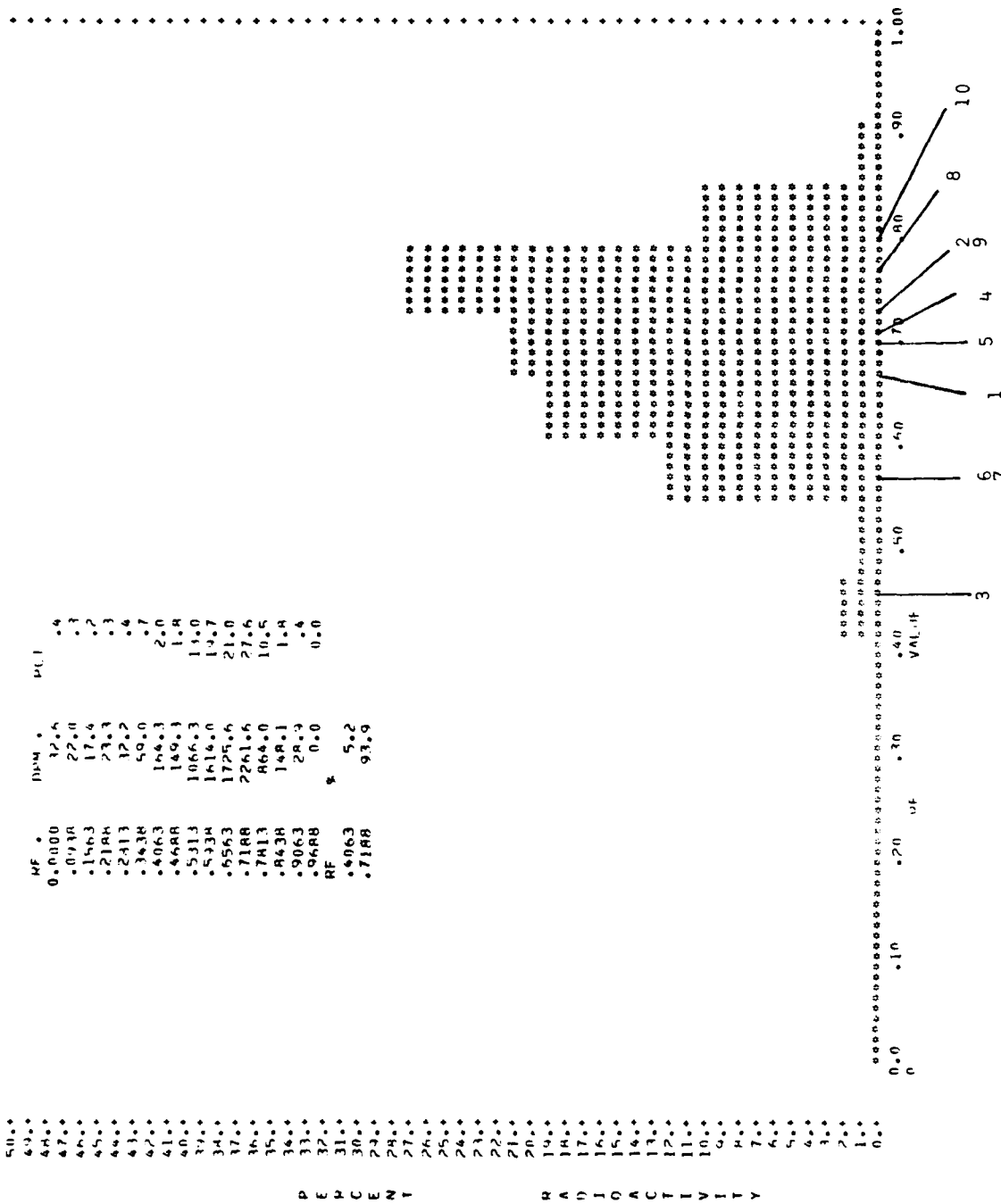
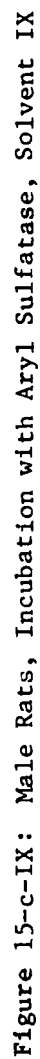


Figure 15-c-I: Male Rats, Incubation with Aryl Sulfatase, Solvent I

[illegible]

4274P SOLVENT 1 NO 5

RF	DM	PC
0.0000	213.7	2.0
.0434	93.0	.8
.1563	47.2	.8
.2144	94.4	.8
.2413	106.5	.9
.3434	135.3	1.2
.4053	420.4	3.7
.4684	568.6	4.4
.5313	435.3	4.1
.5934	1074.7	4.6
.6563	1939.8	15.8
.7184	3930.1	34.1
.7813	1463.5	16.2
.8438	417.8	1.6
.9063	50.4	.4
.9684	0.0	0.0
RF	%	
0.0000	3.6	
.4684	15.8	
.7184	80.6	

P E H C E N T

K A D I D A C T I V I Y

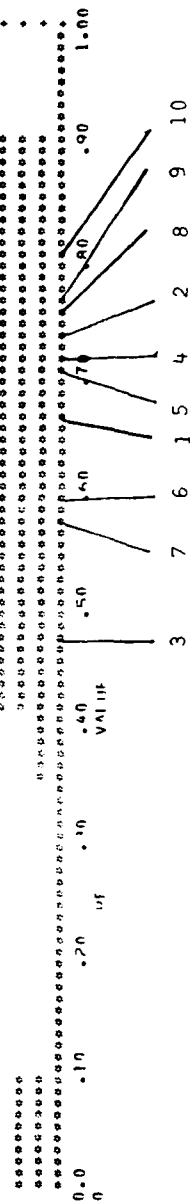


Figure 15-d-I: Female Rats, Incubation with Water, Solvent I

4276H SOLVENT Q NO 5

50.0	0.0000	2173.3	22.7
49.0	.0374	1252.9	12.9
48.0	.1563	1124.9	10.4
47.0	.2184	510.2	7.1
46.0	.2413	564.0	5.4
45.0	.3438	750.0	7.2
44.0	.4063	434.4	4.0
43.0	.4688	871.6	4.3
42.0	.5313	636.6	5.1
41.0	.5938	370.4	3.5
40.0	.6563	395.8	4.8
39.0	.7188	399.8	3.8
38.0	.7413	142.4	1.4
37.0	.8438	54.4	.5
36.0	.9063	27.9	.3
35.0	.9688	13.9	.1
34.0	HF		
33.0	0.0000	50.5	
32.0	.4063	30.5	
31.0	.7188	9.9	

P E M C E N Y

M A D O C V I Y



Figure 15-d-IX: Female Rats, Incubation with Water, Solvent IX

4276H SOLVENT 1 NO 6

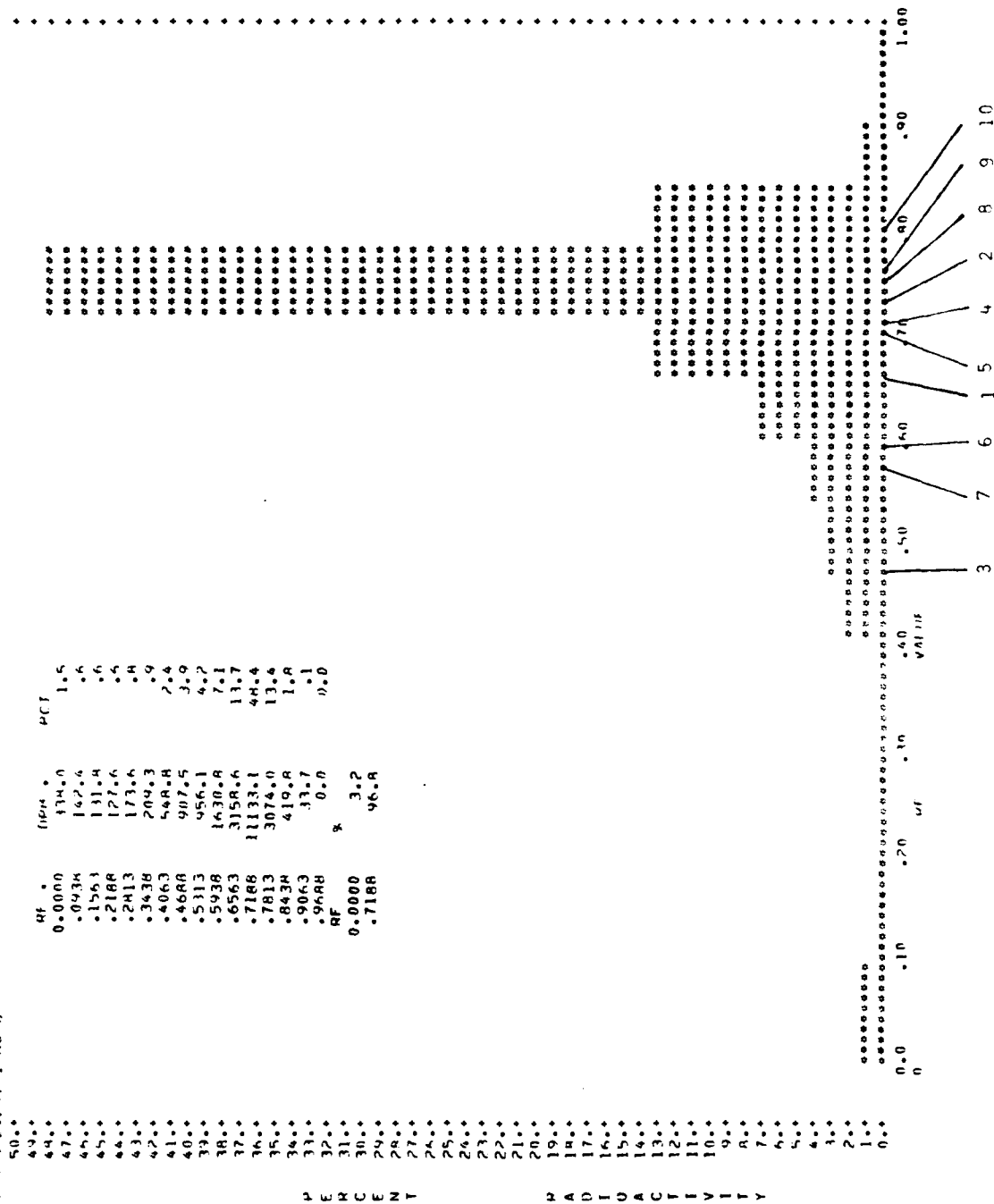


Figure 15-e-I: Female Rats, Incubation with β -Glucuronidase, Solvent I

4274H SOLVENT I NO H

HF	DDM	PC
0.0000	157.4	2.1
0.0318	66.7	.9
0.1563	54.6	.4
0.2148	64.2	.9
0.2413	78.2	1.0
0.3478	121.1	1.5
0.4063	342.6	4.5
0.4588	326.4	4.3
0.5313	390.3	5.1
0.5938	1025.5	13.5
0.6563	1150.0	15.2
0.7188	1707.0	22.5
0.7413	1509.3	19.9
0.8438	522.1	6.9
0.9063	61.6	.8
0.9688	1.1	.0
AF		
0.0000	3.7	
0.4063	12.2	
0.7188	84.0	

P E M C E N T

R A D I U A C I T Y

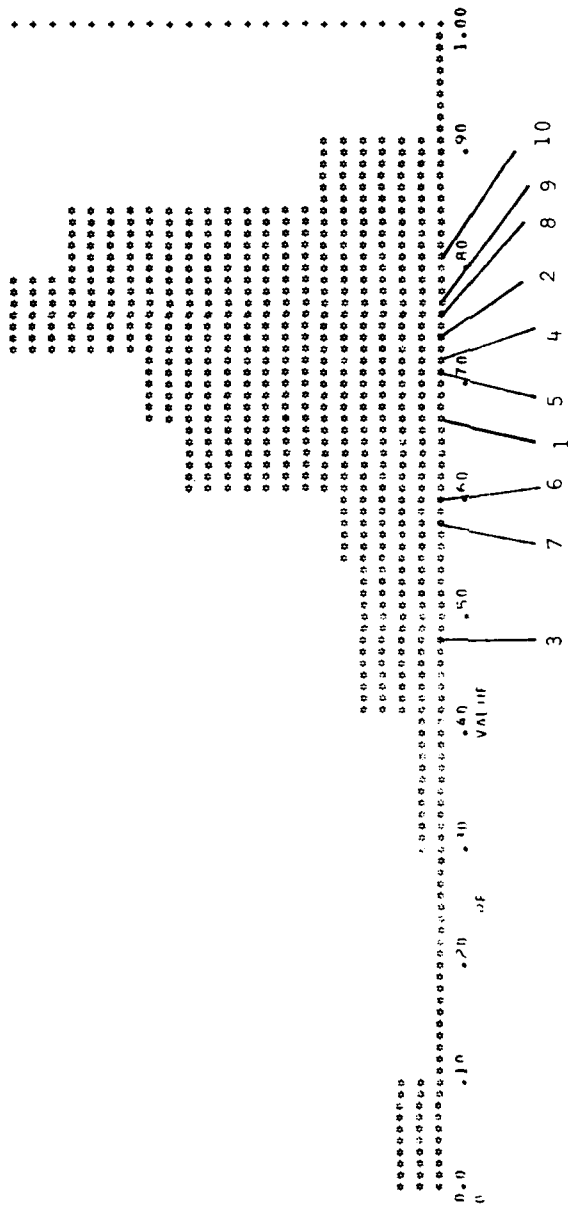


Figure 15-f-I: Female Rats, Incubation with Aryl Sulfatase, Solvent I

4274H SOLVENT 9 NO H

P	50.0	0.0000	2042.1	25.6
E	49.0	0.044	1040.4	12.8
R	48.0	1.563	1407.3	17.3
C	47.0	2.188	468.1	5.6
F	46.0	2.813	454.9	5.0
N	45.0	3.438	404.1	6.4
T	44.0	4.063	518.6	7.5
	43.0	4.688	608.8	4.4
	42.0	5.313	390.8	3.2
	41.0	5.938	262.3	2.5
	40.0	6.563	200.2	2.1
	39.0	7.188	173.7	2.1
	38.0	7.813	62.5	.8
	37.0	8.438	28.1	.3
	36.0	9.063	15.1	.2
	35.0	9.688	0.0	0.0
	34.0	0.0000	38.4	
	33.0	1.563	33.7	
	32.0	4.688	27.8	

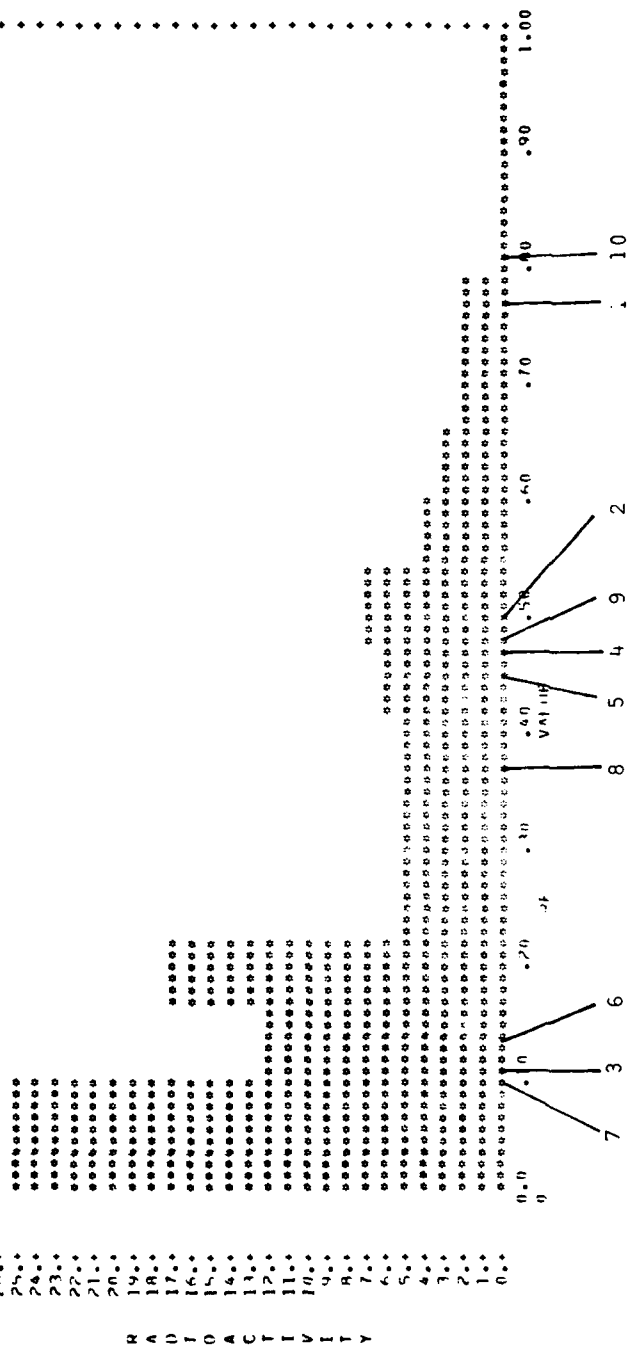


Figure 15-f-IX: Female Rats, Incubation with Aryl Sulfatase, Solvent IX

Figure 16: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Male Rats Treated Orally or Dermally with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 16 follows

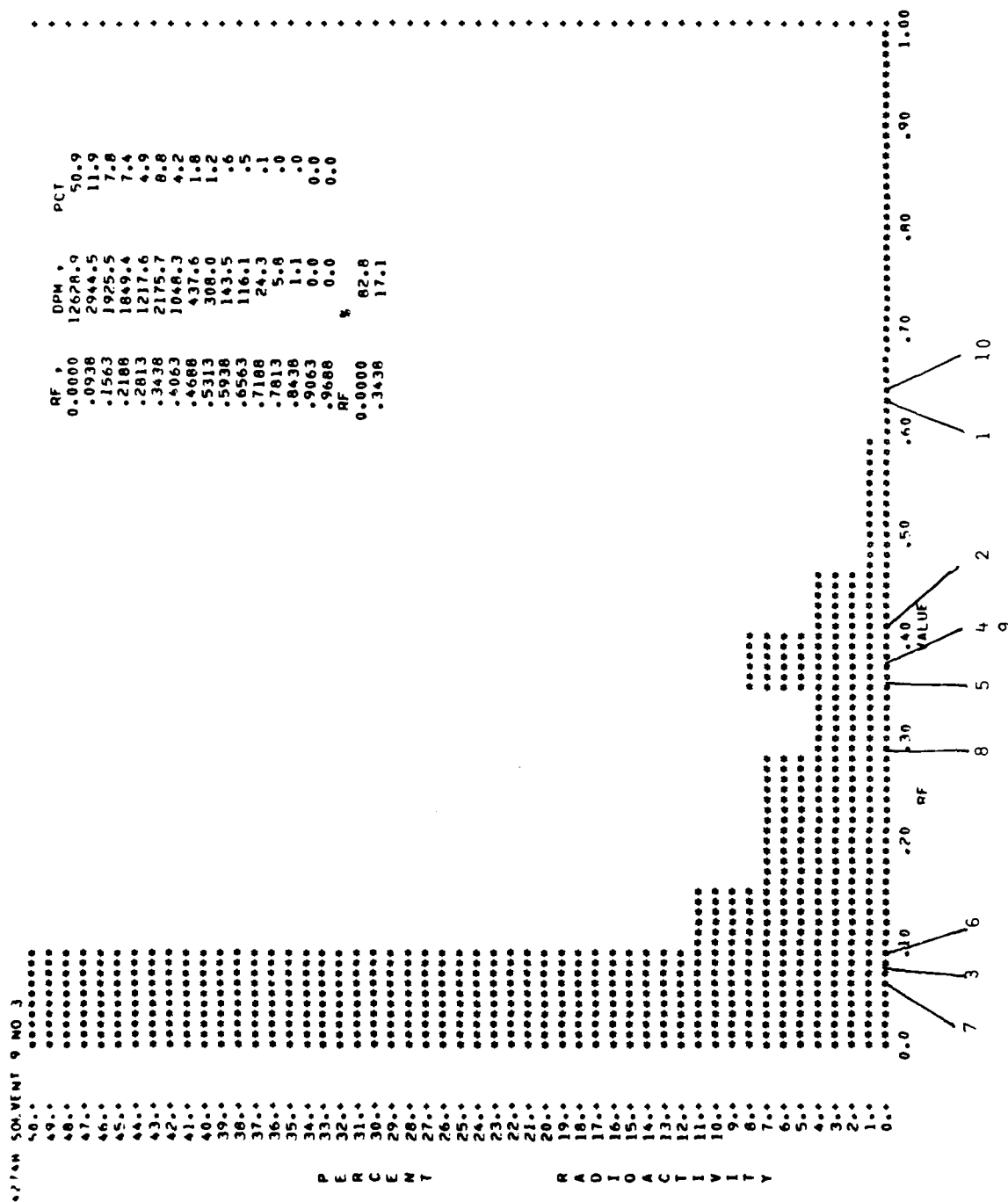


Figure 16-a-IX: Oral Treatment, Incubation with Water, Solvent IX

42748 SOLVENT 1 NO 4

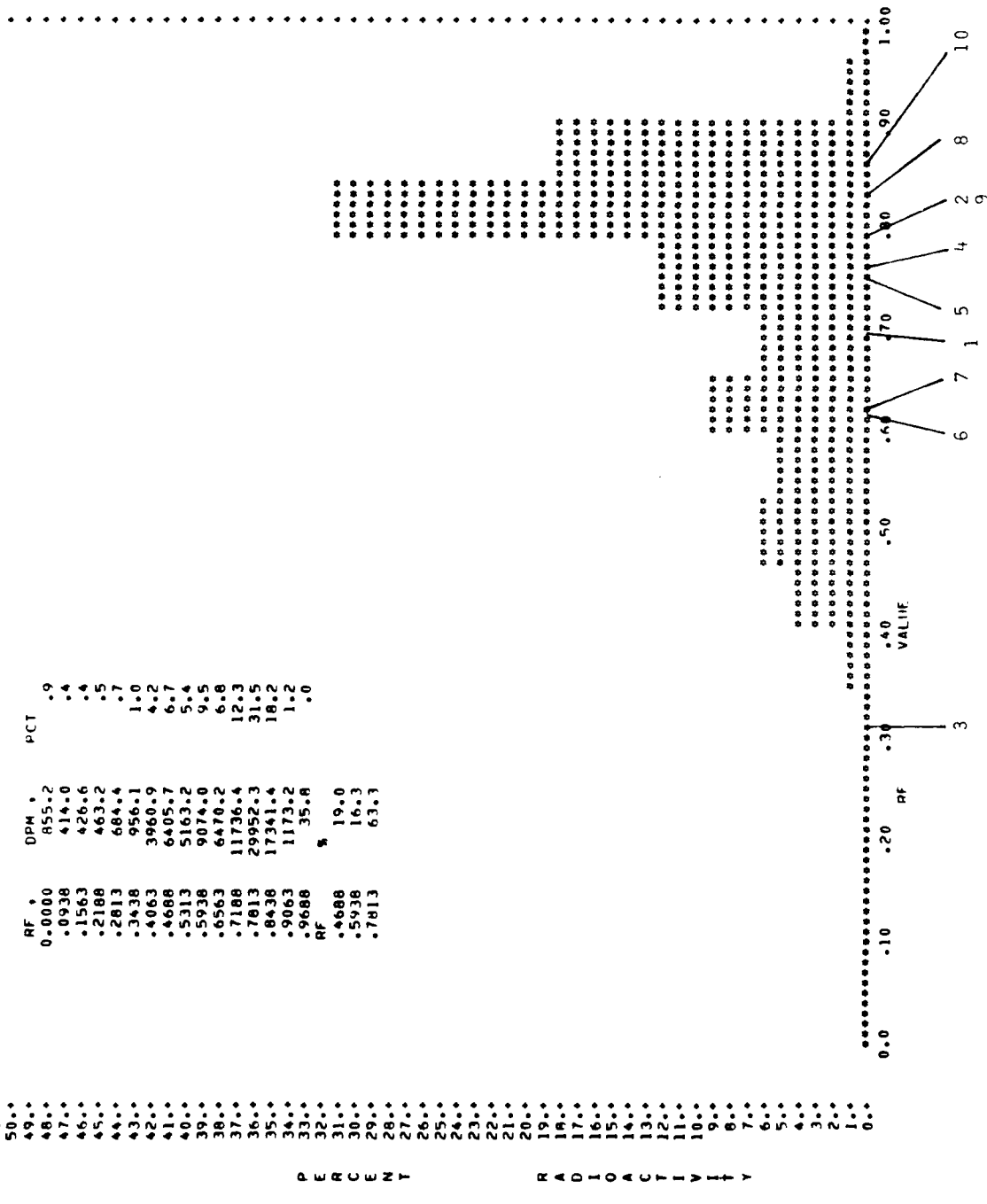


Figure 16-b-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

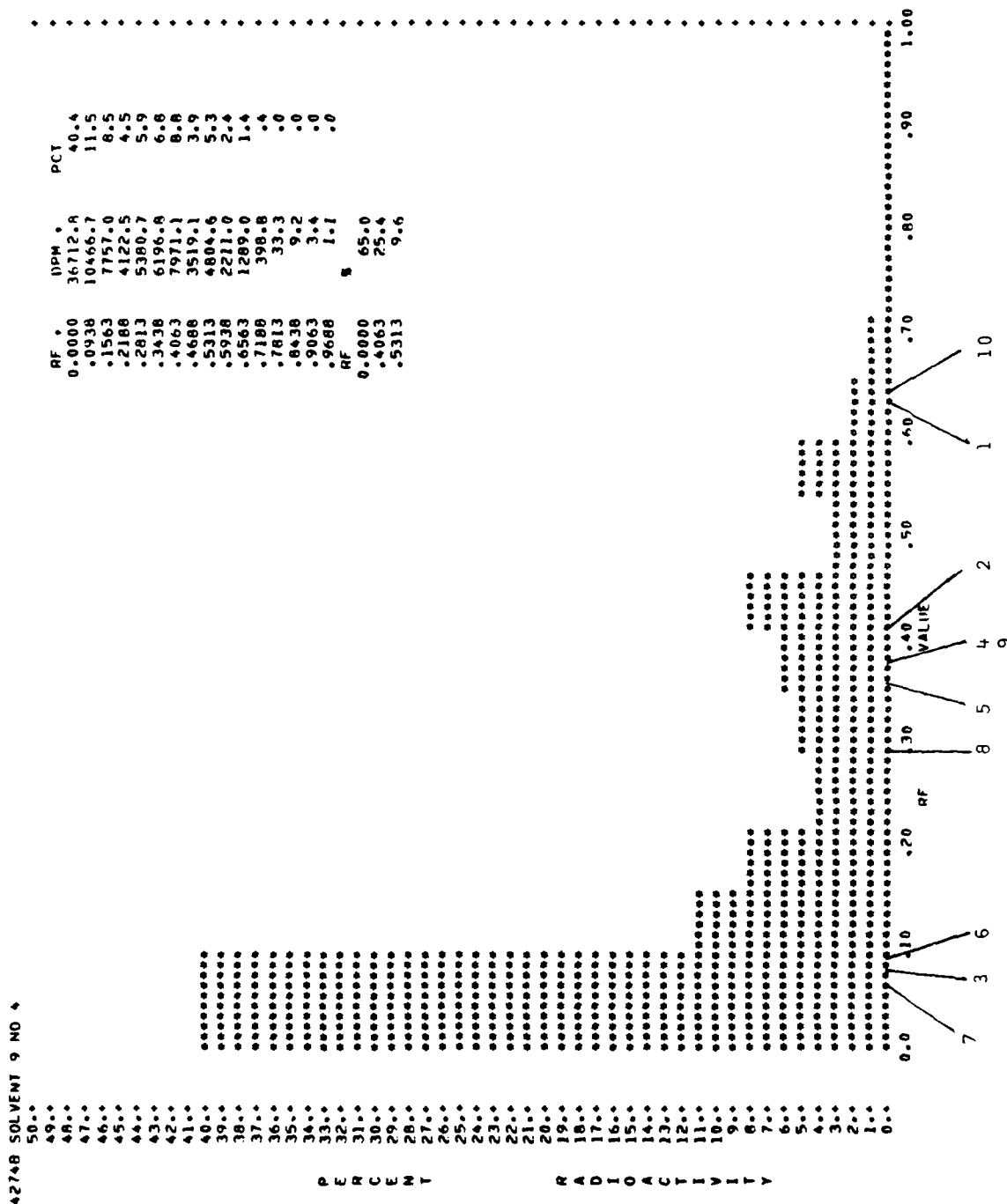


Figure 16-b-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

42748 SOLVENT 1 NO 2

RF	DPM	PCT
0.0000	237.9	1.2
.0938	100.9	.5
.1563	96.6	.5
.2188	143.3	.7
.2813	193.1	.9
.3438	244.8	1.2
.4063	1018.4	4.9
.4688	1165.1	5.6
.5313	1584.5	7.7
.5938	1831.0	8.9
.6563	1901.1	9.2
.7188	3868.2	18.7
.7813	5361.1	25.9
.8438	2633.1	12.7
.9063	268.2	1.3
.9688	25.3	.1
RF	%	
0.0000	2.1	
.7813	97.9	

P E R C E N T

R A D I O A C T I V I T Y

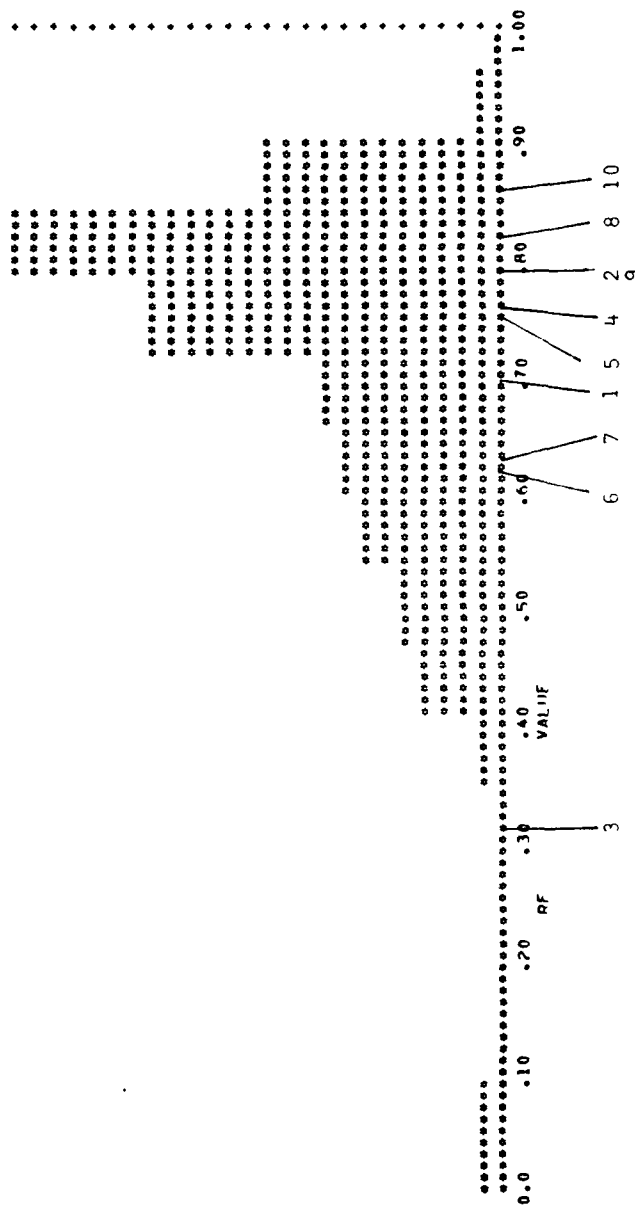


Figure 16-c-I: Dermal Application, Incubation with Water, Solvent I

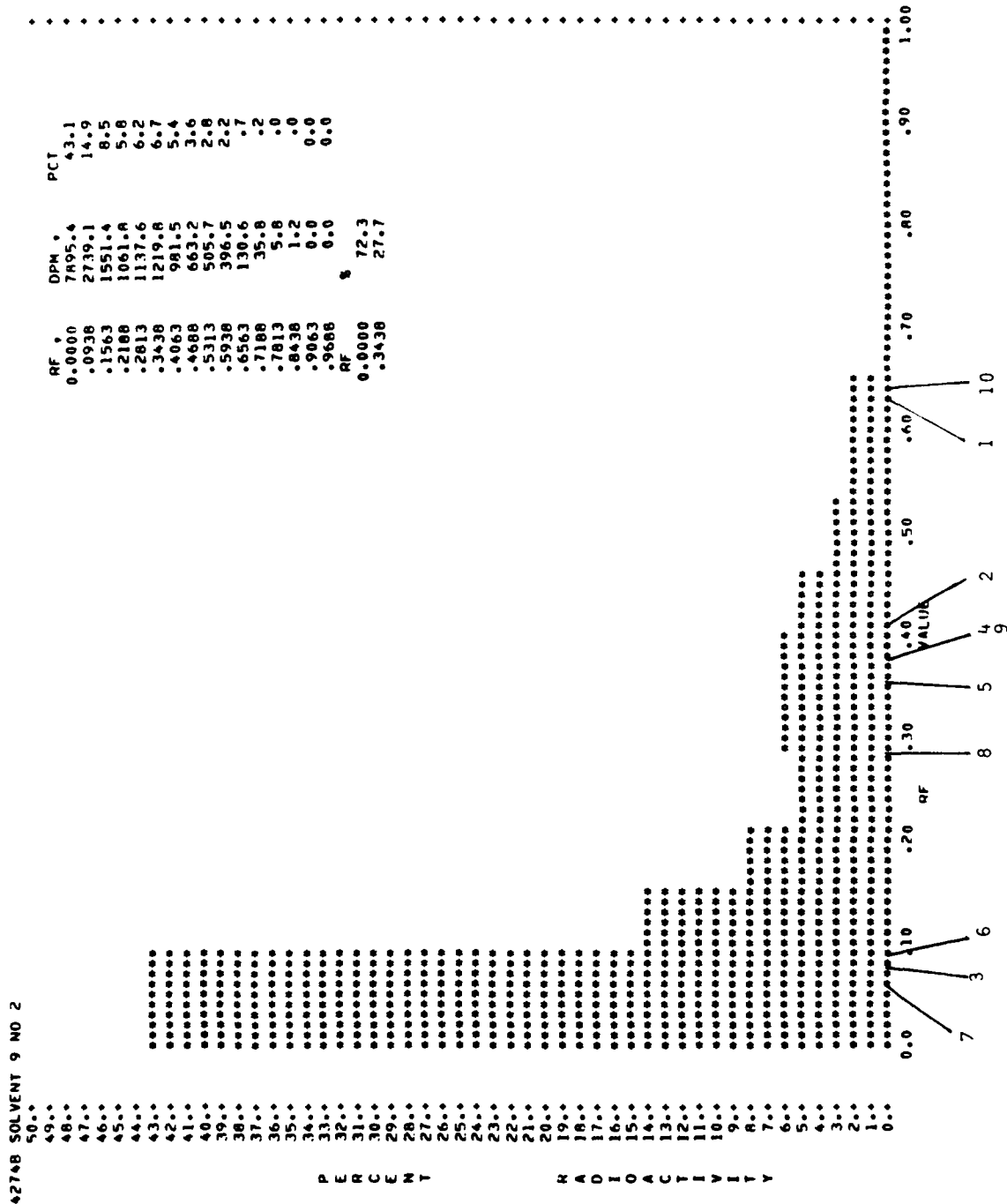


Figure 16-c-IX: Dermal Application, Incubation with Water, Solvent IX

42748 SOLVENT 1 NO 14

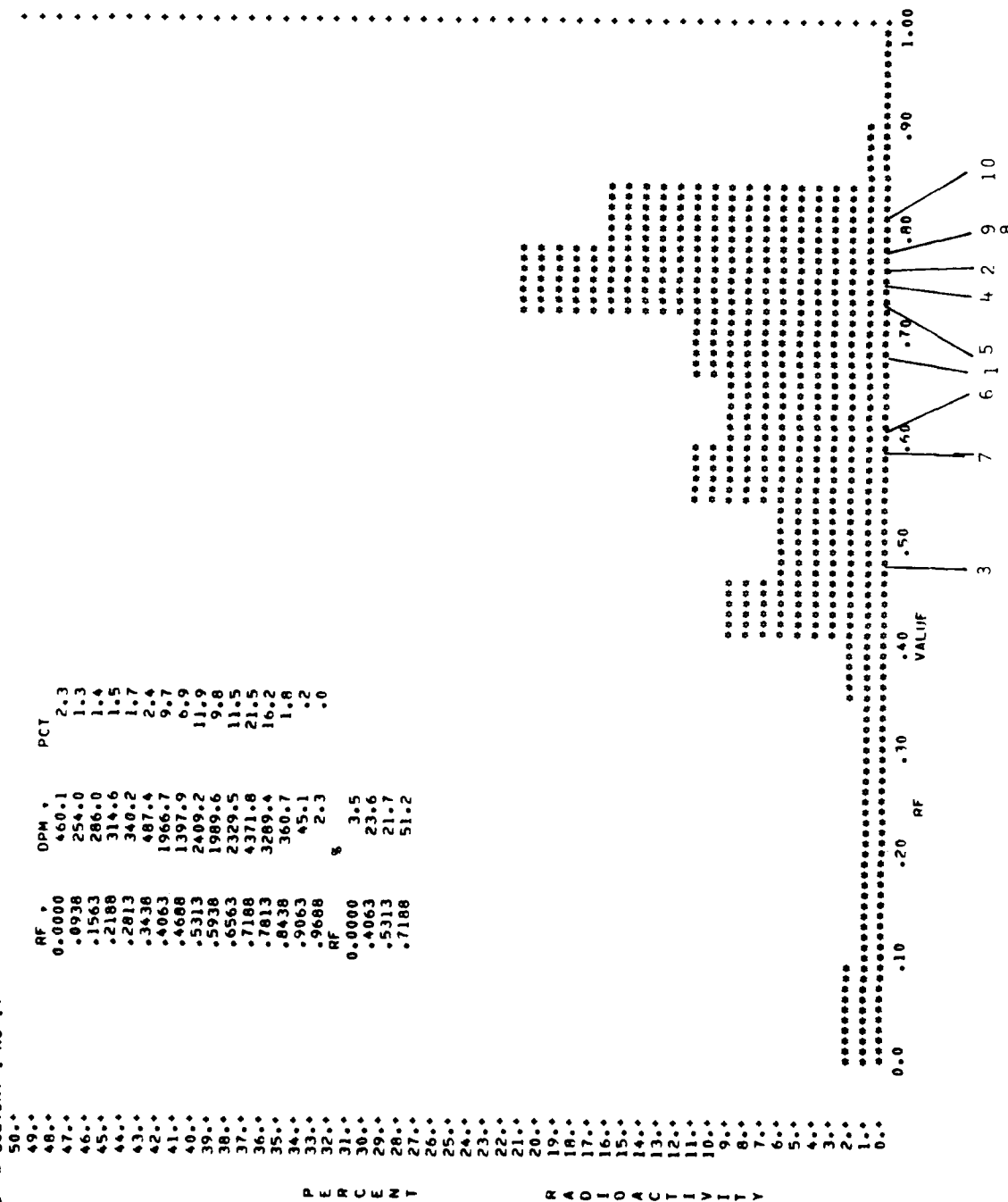


Figure 16-d-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

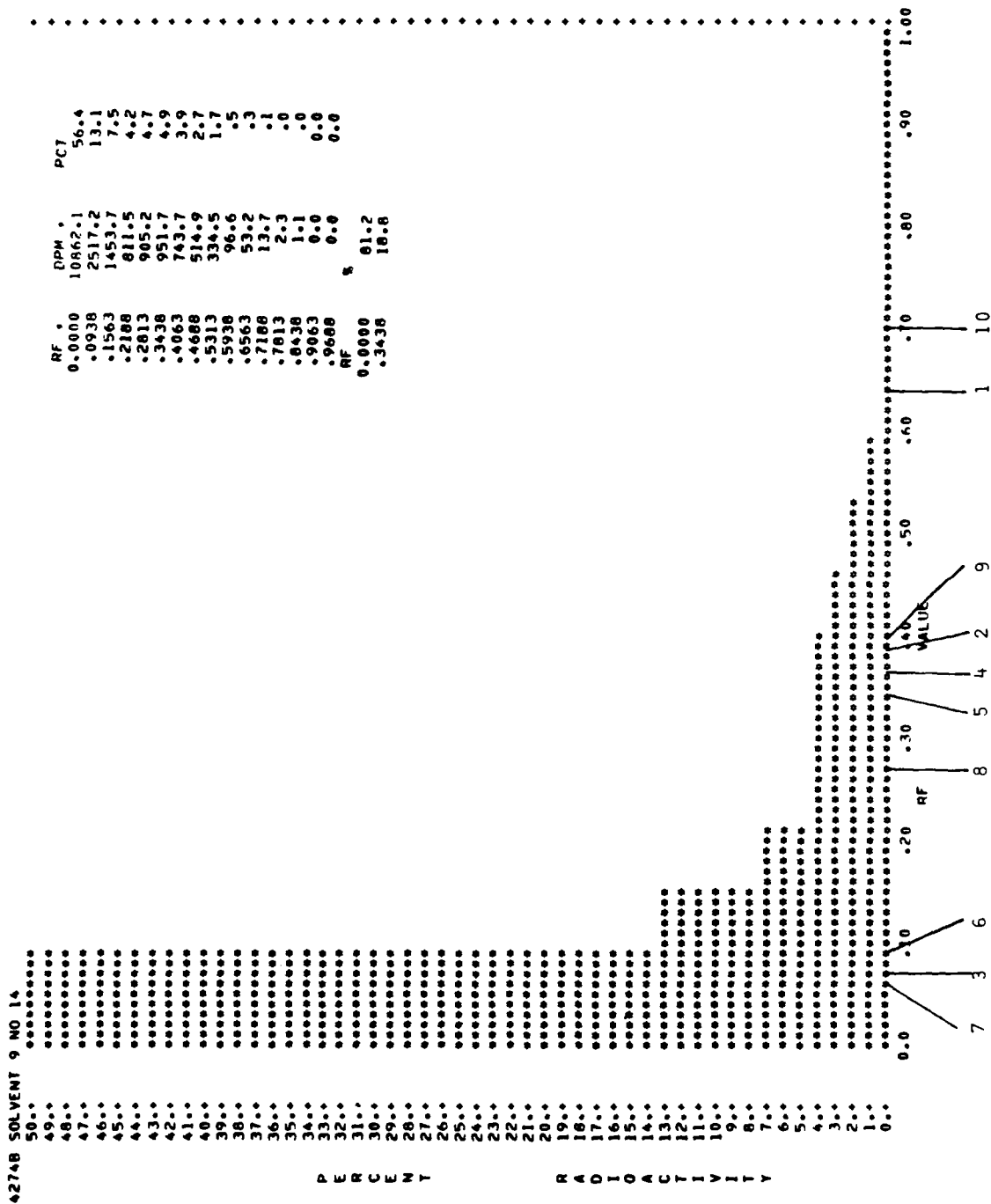


Figure 16-d-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

4274P SOLVENT 1 NO 3 JAN 27

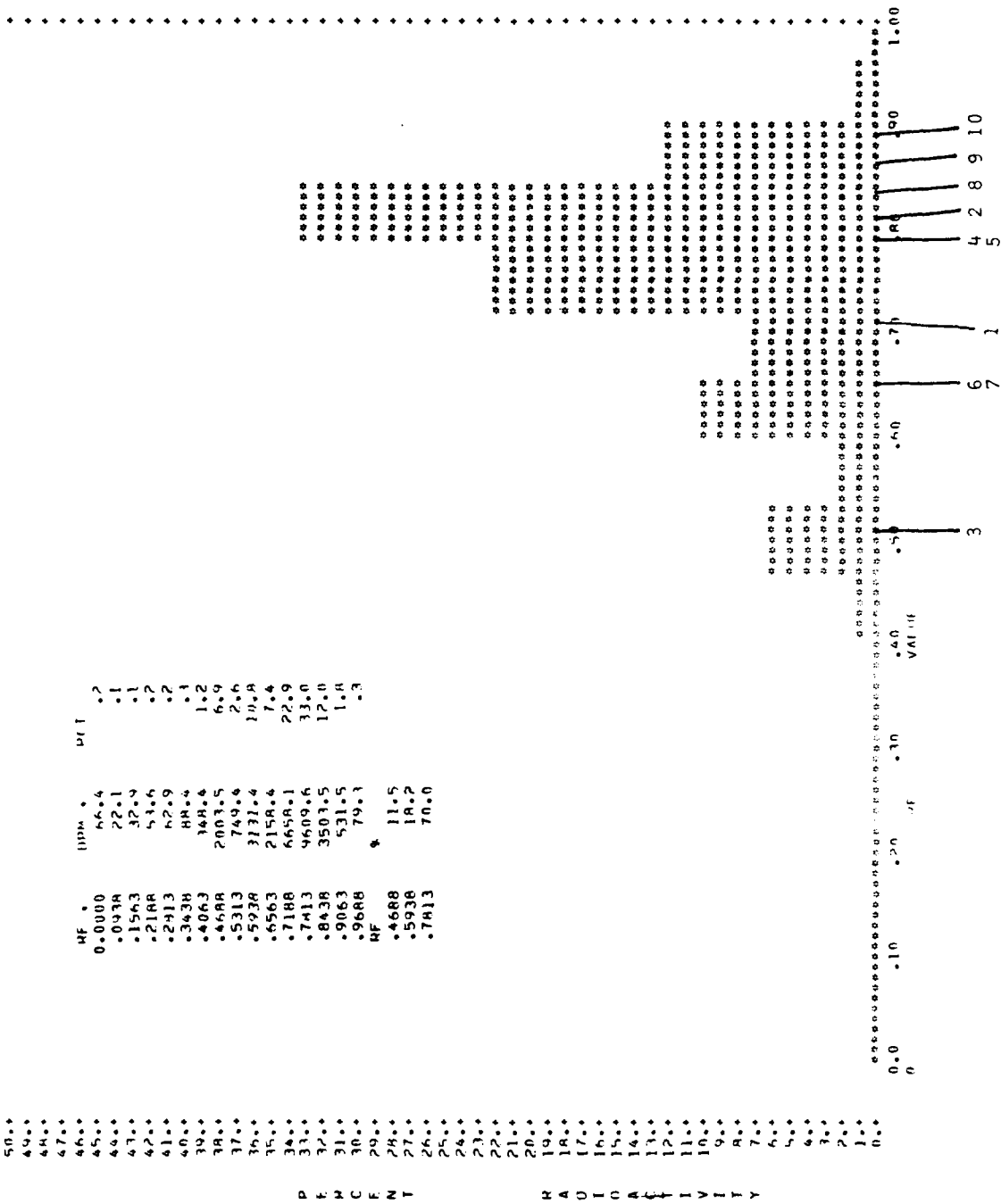


Figure 16-e-I: Oral Treatment, Incubation with Water, Solvent I

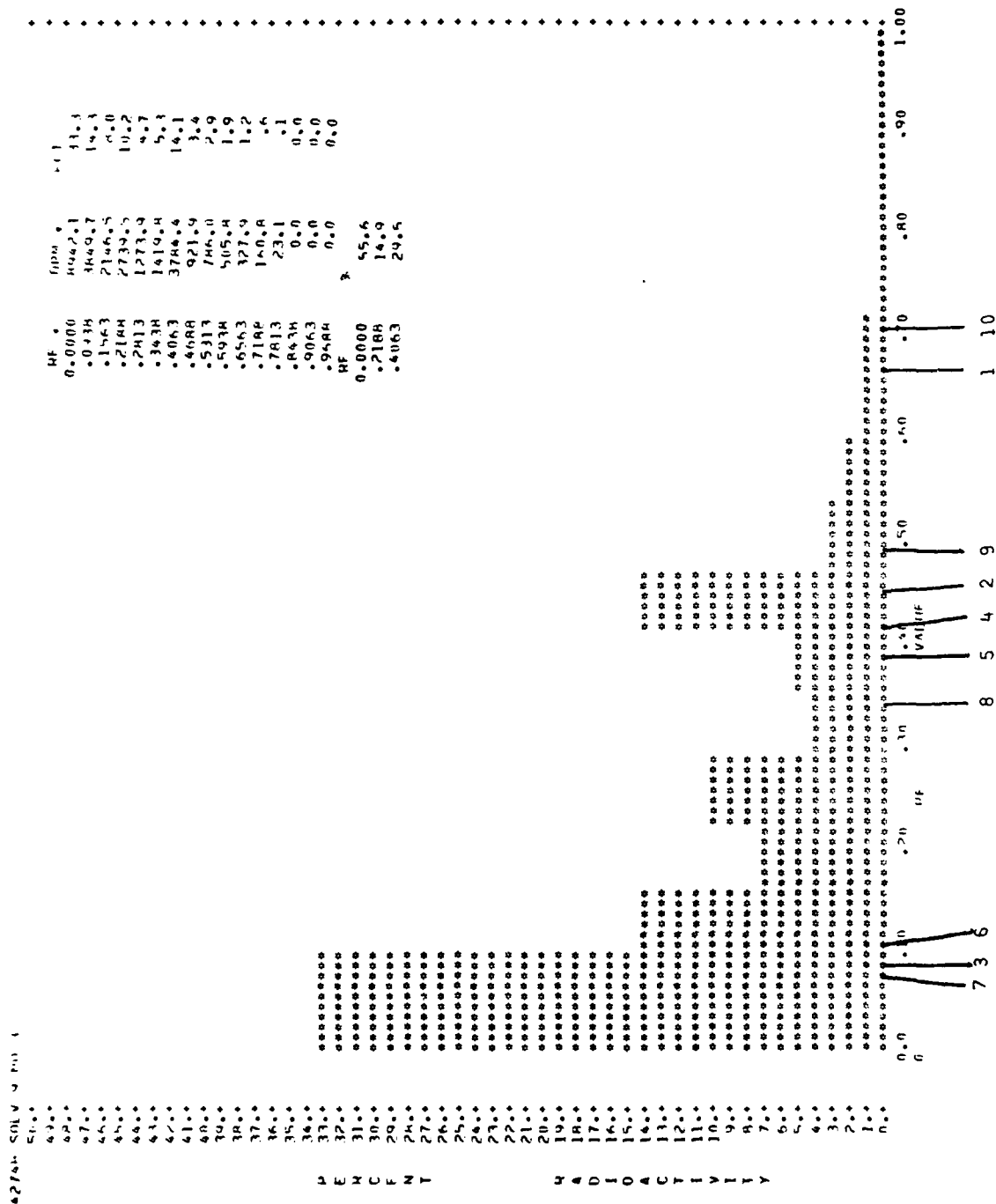
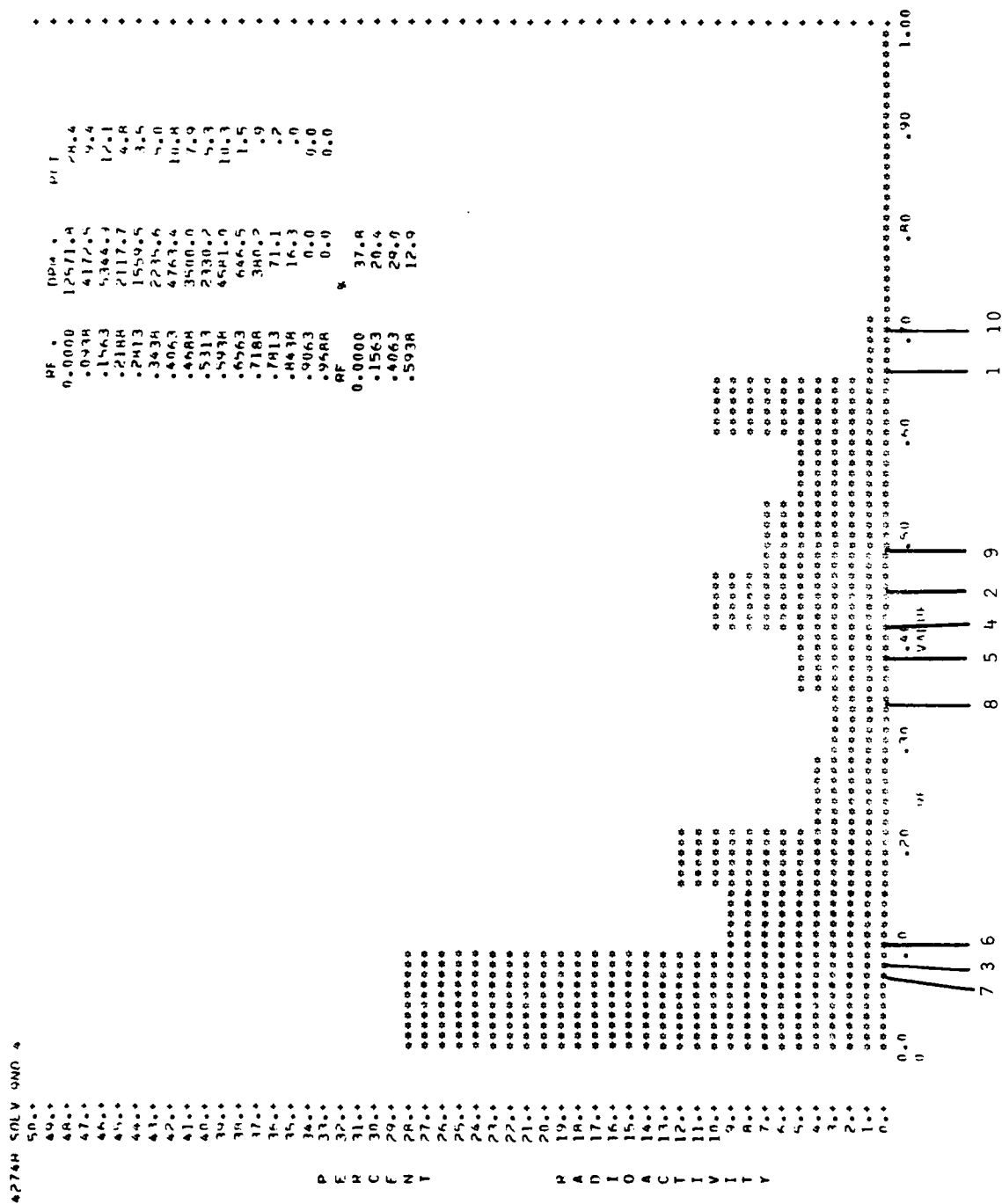


Figure 16-e-IX: Oral Treatment, Incubation with Water, Solvent IX



42744 SOLVENT NO. 1 00.1

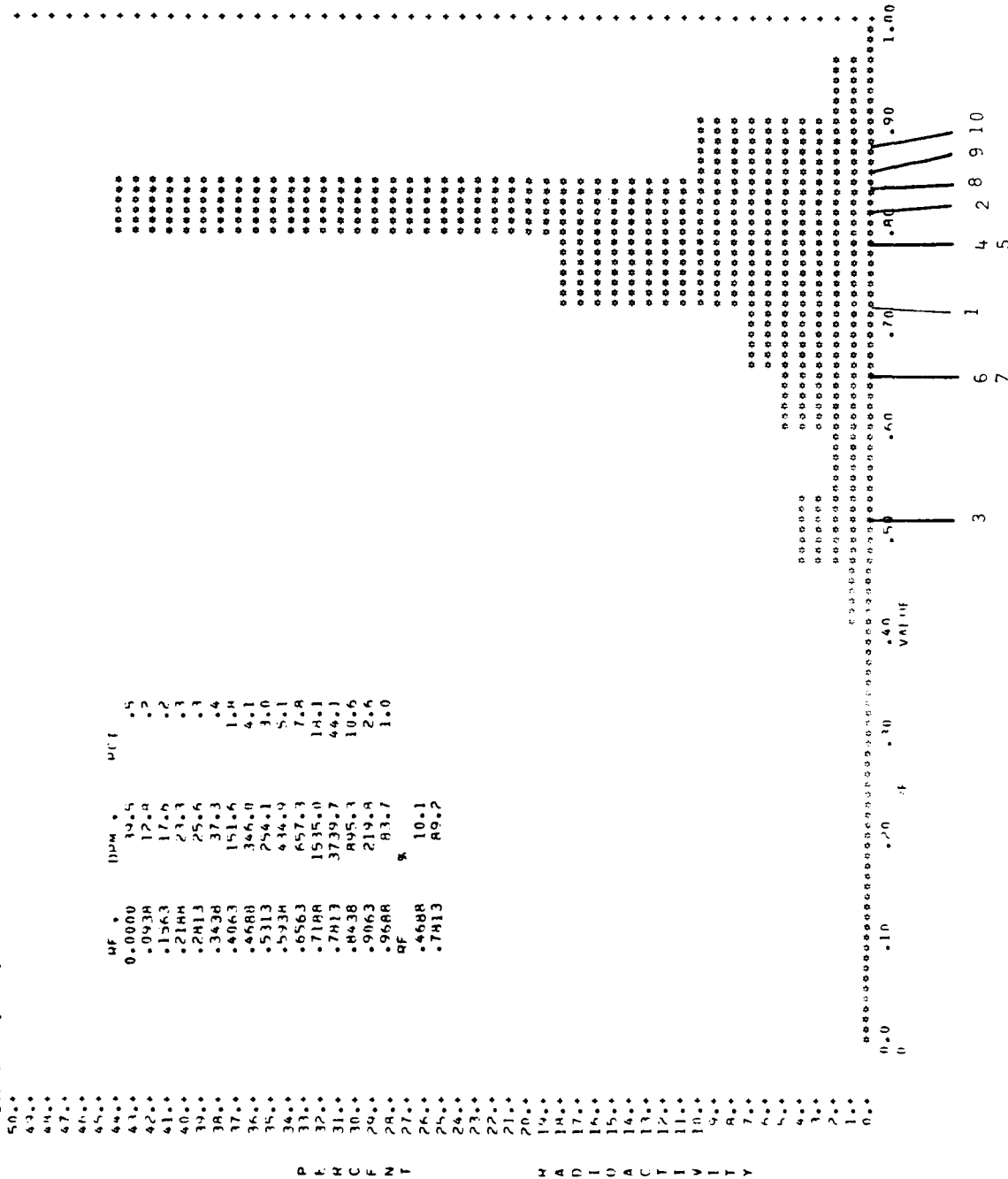


Figure 16-g-I: Dermal Application, Incubation with Water, β -Glucuronidase, Solvent I

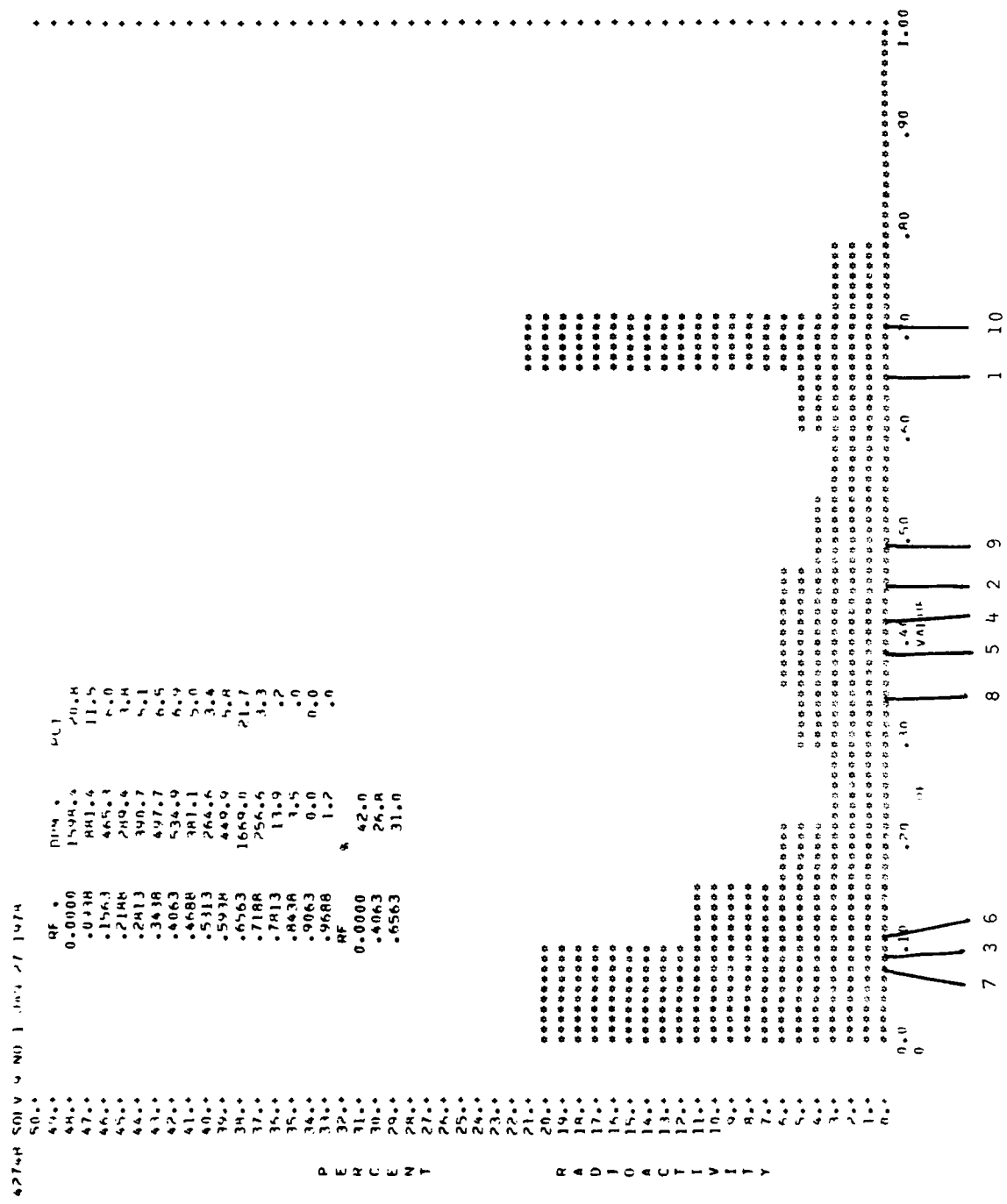


Figure 16-g-IX: Dermal Application, Incubation with Water, β -Glucuronidase, Solvent I

Wavelength (nm)	Intensity (a.u.)	Wavelength (nm)	Intensity (a.u.)
40.0	0.0000	40.0	0.0000
41.0	0.0000	41.0	0.0000
42.0	0.0000	42.0	0.0000
43.0	0.0000	43.0	0.0000
44.0	0.0000	44.0	0.0000
45.0	0.0000	45.0	0.0000
46.0	0.0000	46.0	0.0000
47.0	0.0000	47.0	0.0000
48.0	0.0000	48.0	0.0000
49.0	0.0000	49.0	0.0000
50.0	0.0000	50.0	0.0000
51.0	0.0000	51.0	0.0000
52.0	0.0000	52.0	0.0000
53.0	0.0000	53.0	0.0000
54.0	0.0000	54.0	0.0000
55.0	0.0000	55.0	0.0000
56.0	0.0000	56.0	0.0000
57.0	0.0000	57.0	0.0000
58.0	0.0000	58.0	0.0000
59.0	0.0000	59.0	0.0000
60.0	0.0000	60.0	0.0000
61.0	0.0000	61.0	0.0000
62.0	0.0000	62.0	0.0000
63.0	0.0000	63.0	0.0000
64.0	0.0000	64.0	0.0000
65.0	0.0000	65.0	0.0000
66.0	0.0000	66.0	0.0000
67.0	0.0000	67.0	0.0000
68.0	0.0000	68.0	0.0000
69.0	0.0000	69.0	0.0000
70.0	0.0000	70.0	0.0000
71.0	0.0000	71.0	0.0000
72.0	0.0000	72.0	0.0000
73.0	0.0000	73.0	0.0000
74.0	0.0000	74.0	0.0000
75.0	0.0000	75.0	0.0000
76.0	0.0000	76.0	0.0000
77.0	0.0000	77.0	0.0000
78.0	0.0000	78.0	0.0000
79.0	0.0000	79.0	0.0000
80.0	0.0000	80.0	0.0000
81.0	0.0000	81.0	0.0000
82.0	0.0000	82.0	0.0000
83.0	0.0000	83.0	0.0000
84.0	0.0000	84.0	0.0000
85.0	0.0000	85.0	0.0000
86.0	0.0000	86.0	0.0000
87.0	0.0000	87.0	0.0000
88.0	0.0000	88.0	0.0000
89.0	0.0000	89.0	0.0000
90.0	0.0000	90.0	0.0000
91.0	0.0000	91.0	0.0000
92.0	0.0000	92.0	0.0000
93.0	0.0000	93.0	0.0000
94.0	0.0000	94.0	0.0000
95.0	0.0000	95.0	0.0000
96.0	0.0000	96.0	0.0000
97.0	0.0000	97.0	0.0000
98.0	0.0000	98.0	0.0000
99.0	0.0000	99.0	0.0000
100.0	0.0000	100.0	0.0000

Figure 16-h-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

AD-A114 025

MIDWEST RESEARCH INST KANSAS CITY MO
SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM OF 2,4,6-ETC(U)
JUN 81 A M EL-HAWARI, J R HODGSON

F/G 6/20

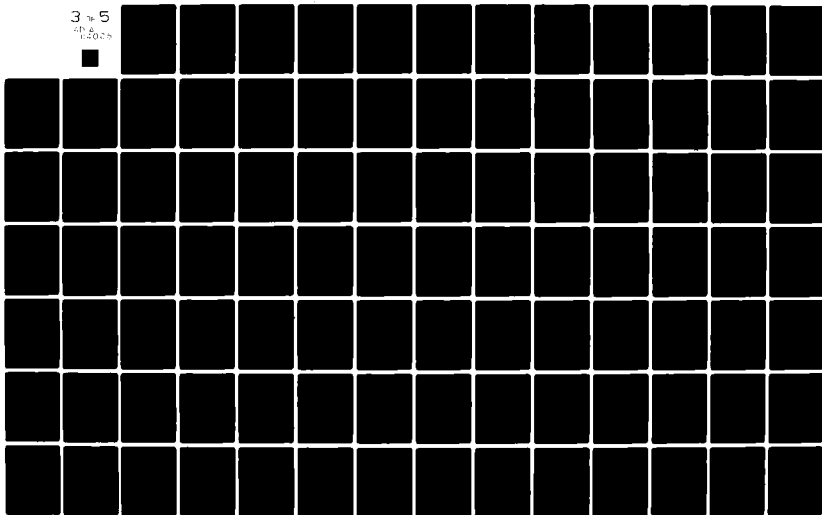
DAMD17-76-C-6066

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UNCLASSIFIED

3 of 5

AD-A114 025



427411 SOLV 4 MD 2

50.0	RF	3674.4	14.4
44.0	0.0000	1452.0	9.8
44.0	.0978	1417.4	7.5
47.0	.1563	760.6	4.0
46.0	.2188	952.3	5.1
44.0	.2813	1290.2	6.8
41.0	.3438	1467.1	10.4
42.0	.4063	1993.0	10.5
41.0	.4688	1670.2	8.8
39.0	.5313	1167.4	6.2
38.0	.5938	1622.7	4.6
37.0	.6563	539.4	2.4
36.0	.7188	37.3	.2
35.0	.7813	1.2	.0
34.0	.8438	2.3	.0
33.0	.9063	0.0	0.0
32.0	.9688		
31.0	RF		
30.0	0.0000	40.6	
29.0	.0688	47.7	
28.0	.1313	11.6	

P E M C E N T

R A D I O A C T I V I T Y

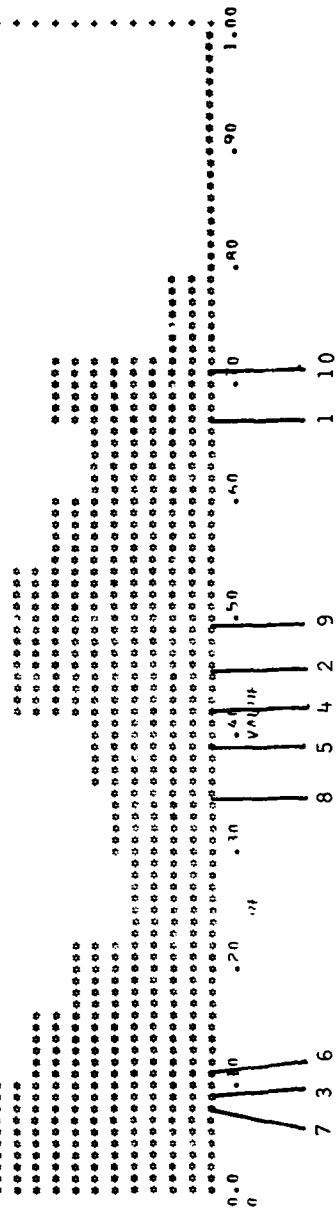


Figure 16-h-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

4274H SOLV 1 NO 7

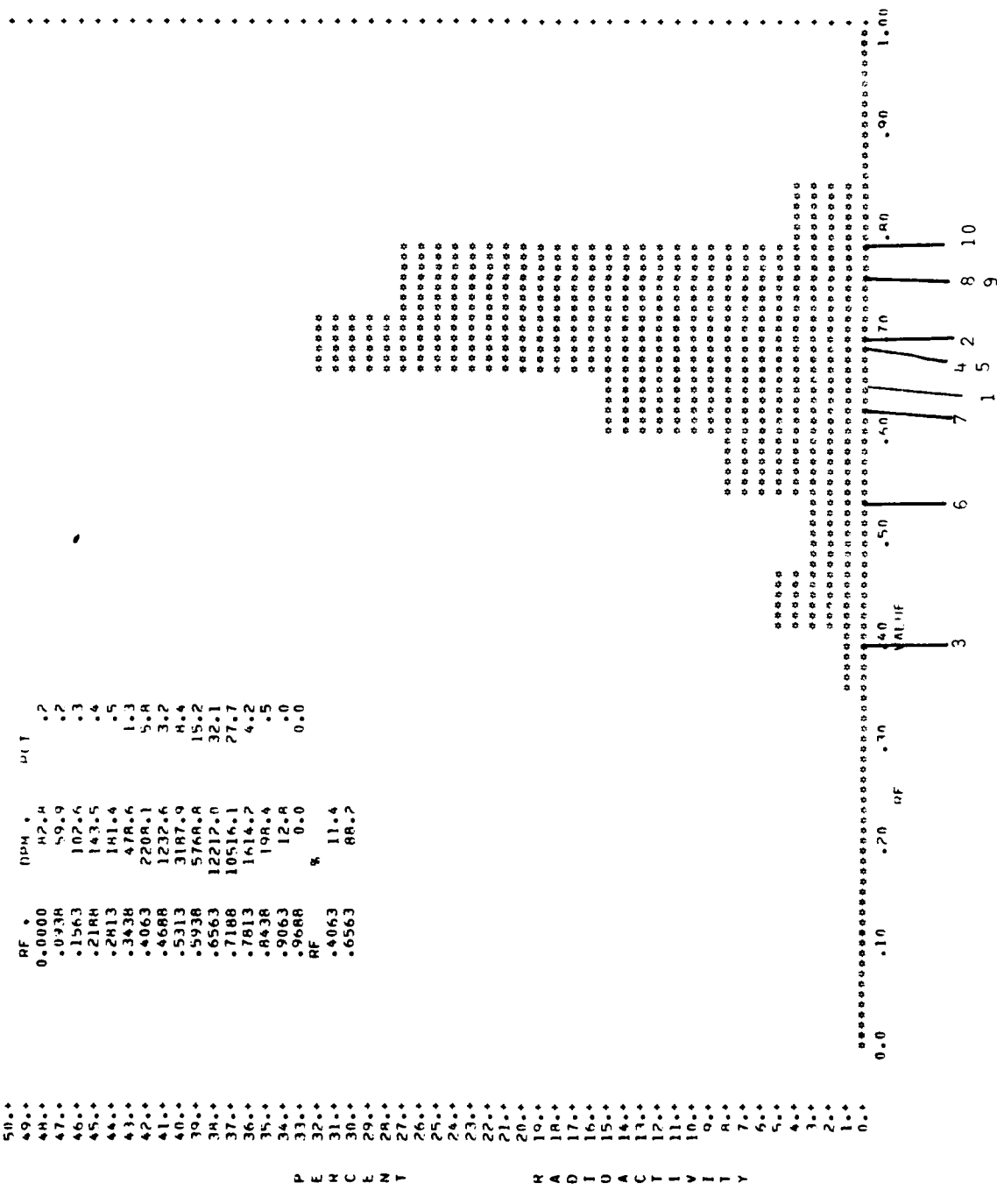


Figure 16-k-I: Oral Treatment, Incubation with Water, Solvent I

4274R SOLV 9 NO 7

50..	RF	DPM	PCT
49..	0.0000	14292.5	40.0
48..	.0938	4650.1	13.0
47..	.1563	3216.3	9.0
46..	.2188	2537.2	7.1
45..	.2813	2201.9	6.2
44..	.3438	4613.7	12.9
43..	.4063	1789.0	5.0
42..	.4688	1004.7	2.8
41..	.5313	785.5	2.2
40..	.5938	379.1	1.1
39..	.6563	188.8	.5
38..	.7188	28.3	.1
37..	.7813	3.5	.0
36..	.8438	0.0	0.0
35..	.9063	0.0	0.0
34..	.9688	0.0	0.0
33..	RF	%	
32..	0.0000	75.4	
31..	.3438	24.6	
30..			
29..			
28..			
27..			
26..			
25..			
24..			
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8..			
7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E W C E M N T R A D I O C T I V I T Y

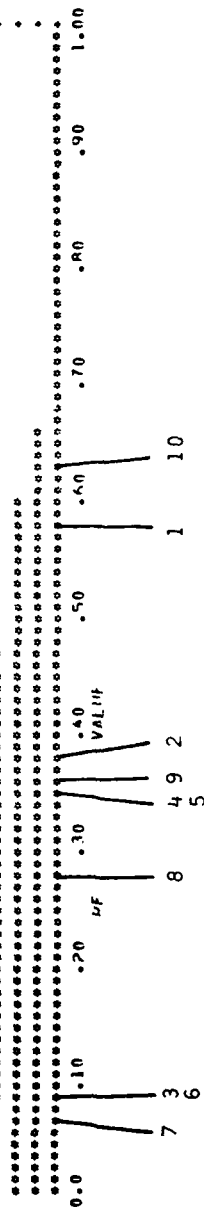


Figure 16-k-IX: Oral Treatment, Incubation with Water, Solvent IX

HF.	DEM.	PC1
50.0	0.0000	
49.9	.219.1	.1
49.8	199.5	.2
49.7	.0938	.2
49.6	163.1	.3
49.5	.1563	.3
49.4	2189	.4
49.3	.2813	.4
49.2	803.0	1.1
49.1	.3438	3.4
49.0	2559.9	3.9
48.9	.4063	7.0
48.8	6680	6.0
48.7	.5313	20.2
48.6	5996.9	45.3
48.5	.5938	11.1
48.4	15275.9	.7
48.3	.6563	.0
48.2	34280.6	0.0
48.1	.7188	
48.0	8366.0	
47.9	.7813	
47.8	511.7	
47.7	.8438	
47.6	.9063	
47.5	19.9	
47.4	.9688	
47.3	.37.0	
47.2	22.0	
47.1	.5313	
47.0	.7188	
46.9	77.2	

SECRET

RADIOACTIVITY

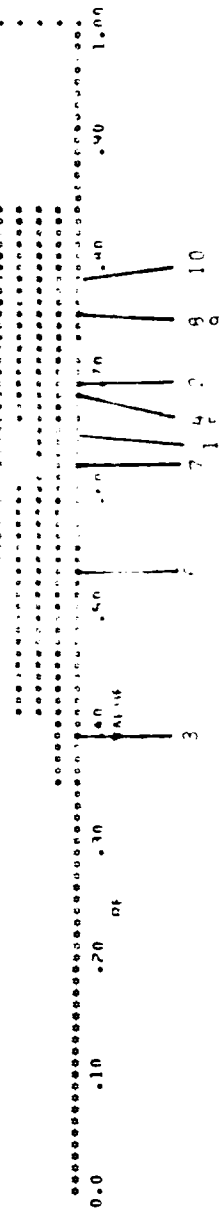


Figure 16-1-I: Oral Treatment, Incubation with α -Glucuronidase, Solvent I

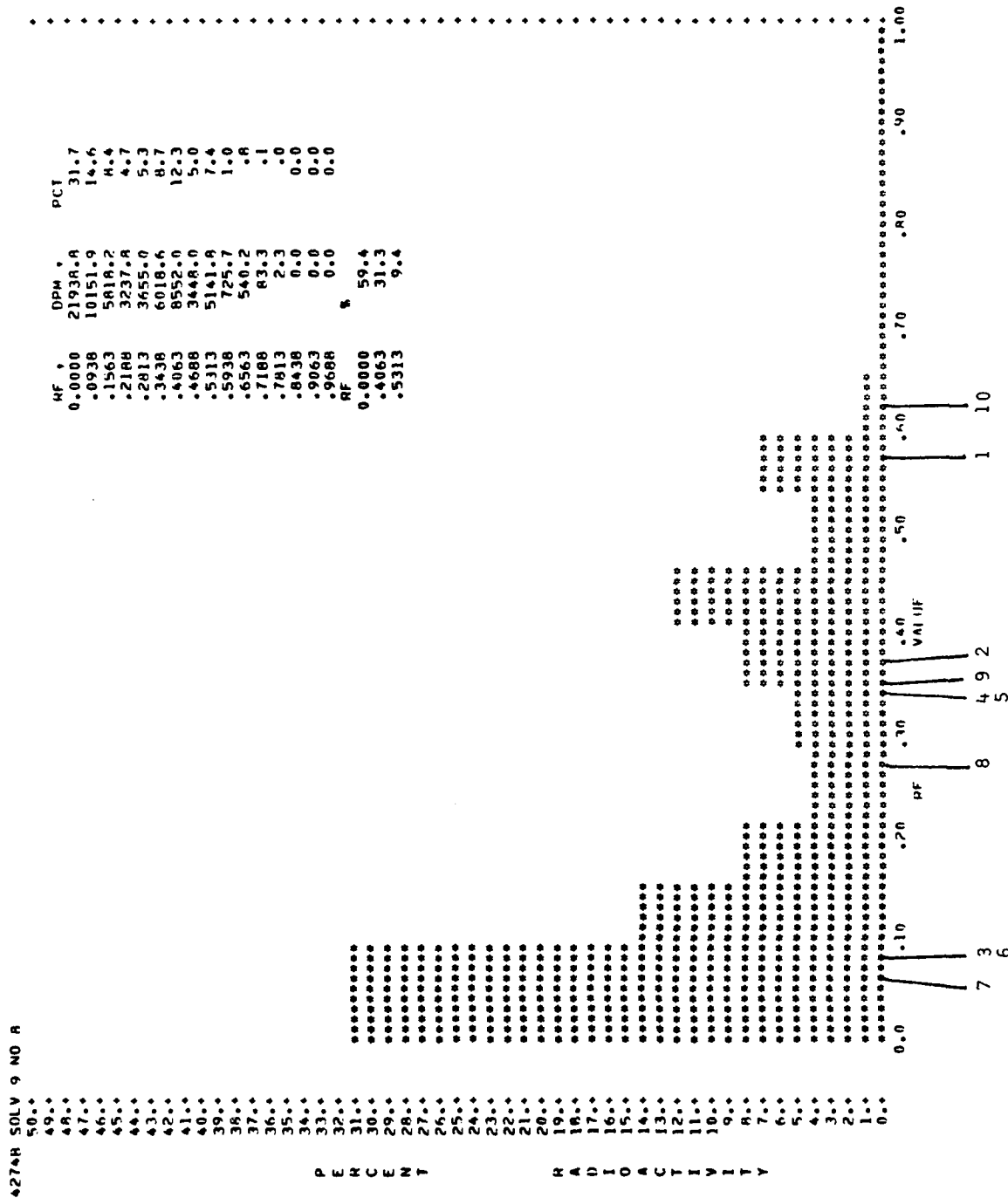


Figure 16-1-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

HF	PPA	WCI
0.000	176.7	1.5
0.934	70.6	1.4
1.563	53.3	1.7
2.108	35.6	1.1
2.241	66.7	1.3
3.334	92.1	1.0
4.063	207.5	4.0
4.688	359.3	7.1
5.313	248.4	7.7
5.934	688.1	13.6
6.563	458.5	17.0
7.188	1627.3	32.3
7.813	446.5	14.9
8.438	47.3	1.0
9.063	4.7	1
9.688	0.0	0.0
RF	%	
0.000	7.2	
4.688	19.9	
7.188	72.9	

Wavelength (nm)	HF (%)	DPH (%)	WCI
500	0.0000	176.7	1.5
490	0.0000	70.6	1.4
480	0.0000	53.3	1.2
470	0.0000	75.6	1.1
460	0.0000	64.7	1.3
450	0.0000	92.1	1.4
440	0.0000	207.5	4.0
430	0.0000	359.3	7.1
420	0.0000	284.4	7.7
410	0.0000	684.1	13.6
400	0.0000	855.5	17.0
390	0.0000	1622.7	32.3
380	0.0000	446.5	8.9
370	0.0000	52.3	1.0
360	0.0000	4.7	0.1
350	0.0000	0.0	0.0
340	0.0000	7.2	
330	0.0000	19.9	
320	0.0000	72.9	
310	0.0000		
300	0.0000		
290	0.0000		
280	0.0000		
270	0.0000		
260	0.0000		
250	0.0000		
240	0.0000		
230	0.0000		
220	0.0000		
210	0.0000		
200	0.0000		
190	0.0000		
180	0.0000		
170	0.0000		
160	0.0000		
150	0.0000		
140	0.0000		
130	0.0000		
120	0.0000		
110	0.0000		
100	0.0000		
90	0.0000		
80	0.0000		
70	0.0000		
60	0.0000		
50	0.0000		
40	0.0000		
30	0.0000		
20	0.0000		
10	0.0000		
0	0.0000		

Figure 16-m-I: Dermal Application, Incubation with Water, Solvent I

4276H SOLVENT V NO 11

PEHCENIT	WADITACFVIT	HF	100%	Wt
43.0	0.0000	0.0000	1646.0	51.4
44.0	0.0030	0.0030	510.6	3.6
45.0	0.0060	0.0060	156.3	4.1
46.0	0.0090	0.0090	21.4	4.8
47.0	0.0120	0.0120	230.9	4.3
48.0	0.0150	0.0150	644.1	12.2
49.0	0.0180	0.0180	1017.6	14.2
50.0	0.0210	0.0210	468.8	3.4
51.0	0.0240	0.0240	531.3	2.6
52.0	0.0270	0.0270	54.8	1.4
53.0	0.0300	0.0300	65.3	1.4
54.0	0.0330	0.0330	71.8	1.4
55.0	0.0360	0.0360	74.3	1.4
56.0	0.0390	0.0390	84.3	1.4
57.0	0.0420	0.0420	90.3	1.4
58.0	0.0450	0.0450	95.8	1.4
59.0	0.0480	0.0480	95.8	1.4
60.0	0.0510	0.0510	95.8	1.4
61.0	0.0540	0.0540	95.8	1.4
62.0	0.0570	0.0570	95.8	1.4
63.0	0.0600	0.0600	95.8	1.4
64.0	0.0630	0.0630	95.8	1.4
65.0	0.0660	0.0660	95.8	1.4
66.0	0.0690	0.0690	95.8	1.4
67.0	0.0720	0.0720	95.8	1.4
68.0	0.0750	0.0750	95.8	1.4
69.0	0.0780	0.0780	95.8	1.4
70.0	0.0810	0.0810	95.8	1.4
71.0	0.0840	0.0840	95.8	1.4
72.0	0.0870	0.0870	95.8	1.4
73.0	0.0900	0.0900	95.8	1.4
74.0	0.0930	0.0930	95.8	1.4
75.0	0.0960	0.0960	95.8	1.4
76.0	0.0990	0.0990	95.8	1.4
77.0	0.1020	0.1020	95.8	1.4
78.0	0.1050	0.1050	95.8	1.4
79.0	0.1080	0.1080	95.8	1.4
80.0	0.1110	0.1110	95.8	1.4
81.0	0.1140	0.1140	95.8	1.4
82.0	0.1170	0.1170	95.8	1.4
83.0	0.1200	0.1200	95.8	1.4
84.0	0.1230	0.1230	95.8	1.4
85.0	0.1260	0.1260	95.8	1.4
86.0	0.1290	0.1290	95.8	1.4
87.0	0.1320	0.1320	95.8	1.4
88.0	0.1350	0.1350	95.8	1.4
89.0	0.1380	0.1380	95.8	1.4
90.0	0.1410	0.1410	95.8	1.4
91.0	0.1440	0.1440	95.8	1.4
92.0	0.1470	0.1470	95.8	1.4
93.0	0.1500	0.1500	95.8	1.4
94.0	0.1530	0.1530	95.8	1.4
95.0	0.1560	0.1560	95.8	1.4
96.0	0.1590	0.1590	95.8	1.4
97.0	0.1620	0.1620	95.8	1.4
98.0	0.1650	0.1650	95.8	1.4
99.0	0.1680	0.1680	95.8	1.4
100.0	0.1710	0.1710	95.8	1.4

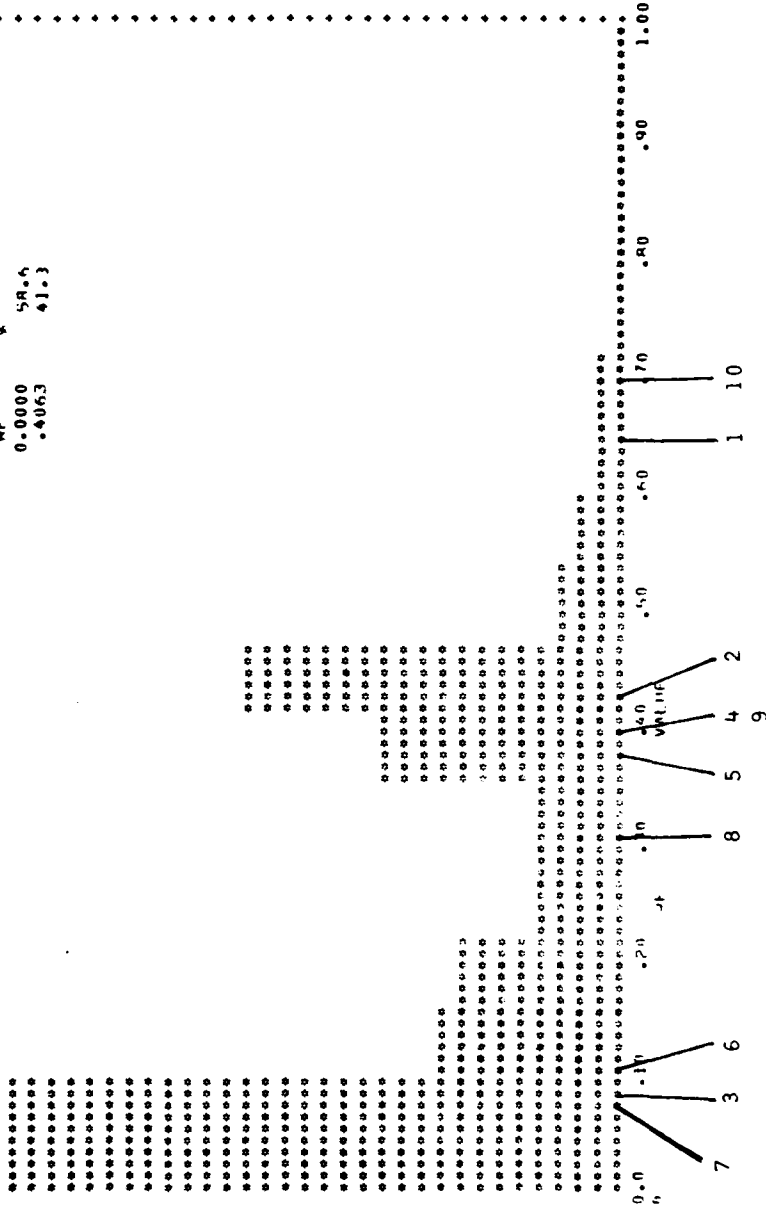


Figure 16-m-IX: Dermal Application, Incubation with Water, Solvent IX

42744 SOLVENT I NO 14

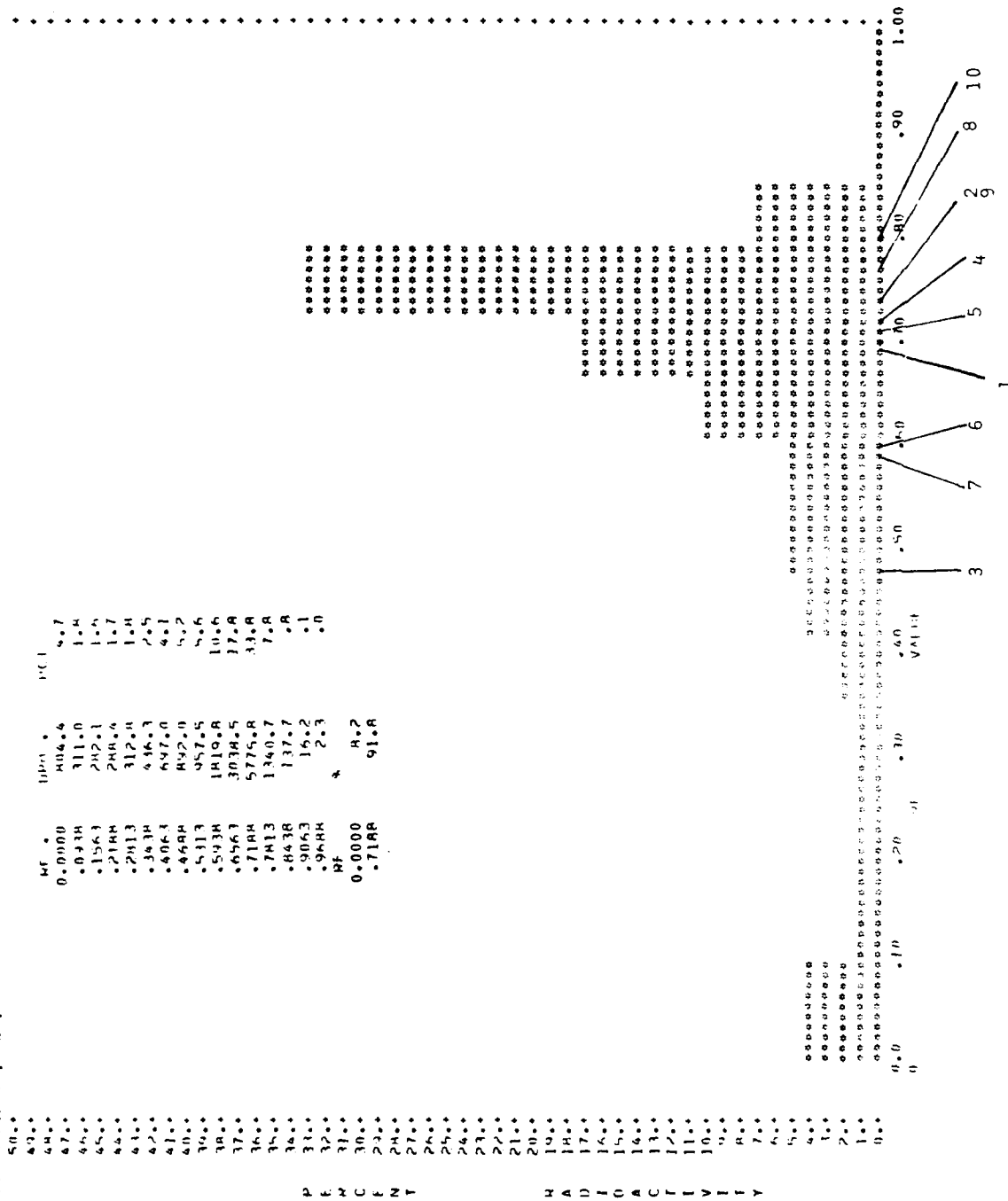


Figure 16-n-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

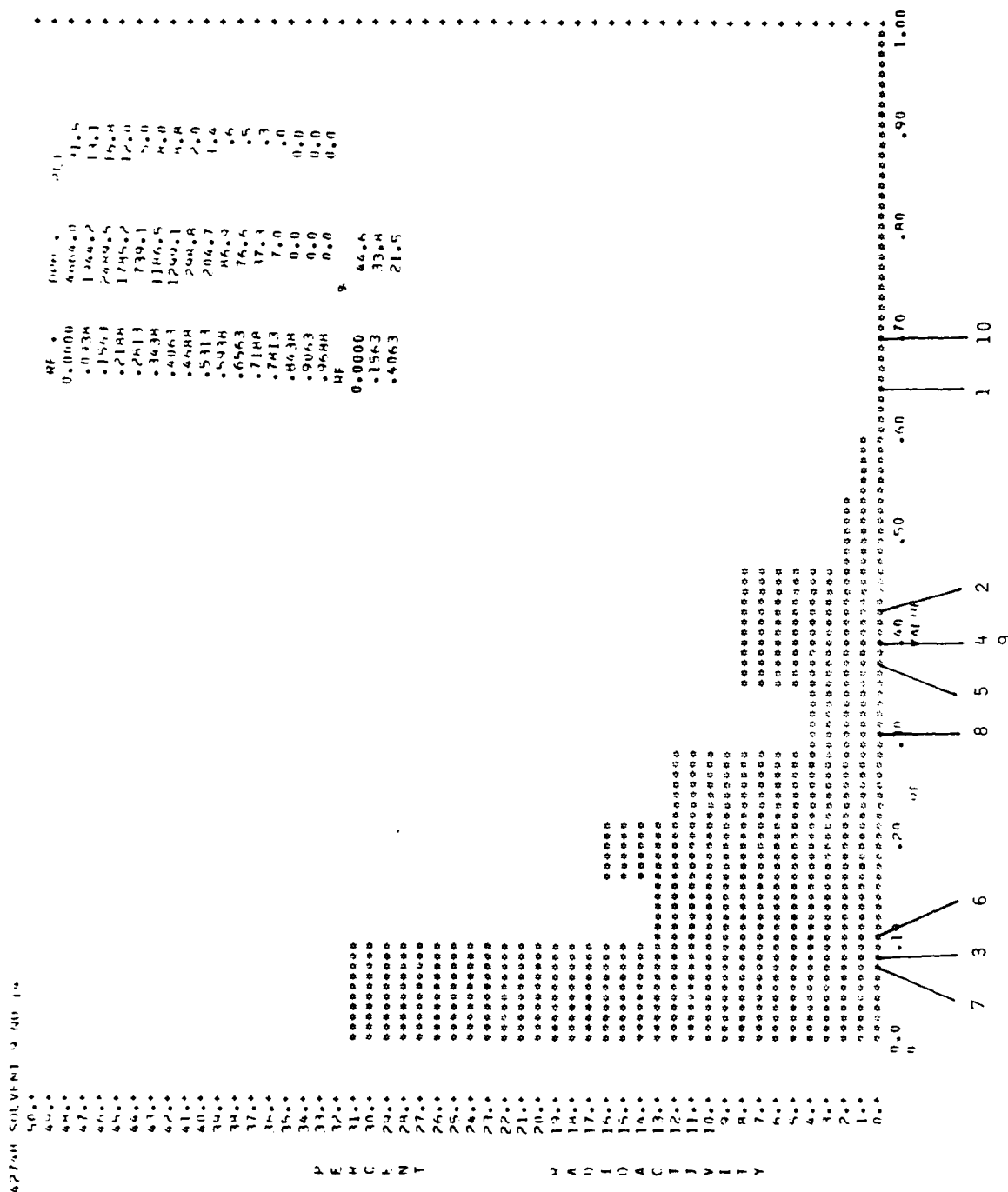


Figure 17: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Female Rats Treated Orally or Dermal with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 17 follows

4274B SOLVENT 1 NO 9

50.0	RF	NP4	PCT
49.0	0.0000	995.4	1.5
48.0	.0938	452.9	.7
47.0	.1563	485.1	.7
46.0	.2188	557.5	.9
45.0	.2813	700.0	1.1
44.0	.3438	888.5	1.4
43.0	.4063	2538.7	3.9
42.0	.4688	3459.9	5.3
41.0	.5313	4167.8	6.4
40.0	.5938	10562.1	16.2
39.0	.6563	7976.9	12.2
38.0	.7188	16482.6	25.2
37.0	.7813	13509.4	20.7
36.0	.8438	2258.6	3.5
35.0	.9063	287.4	.4
34.0	.9688	30.1	.0
33.0	RF		
32.0	0.0000	2.2	
31.0	.5938	47.9	
30.0	.7188	49.8	

P E R C E N T

R A D I O A C T I V I T Y

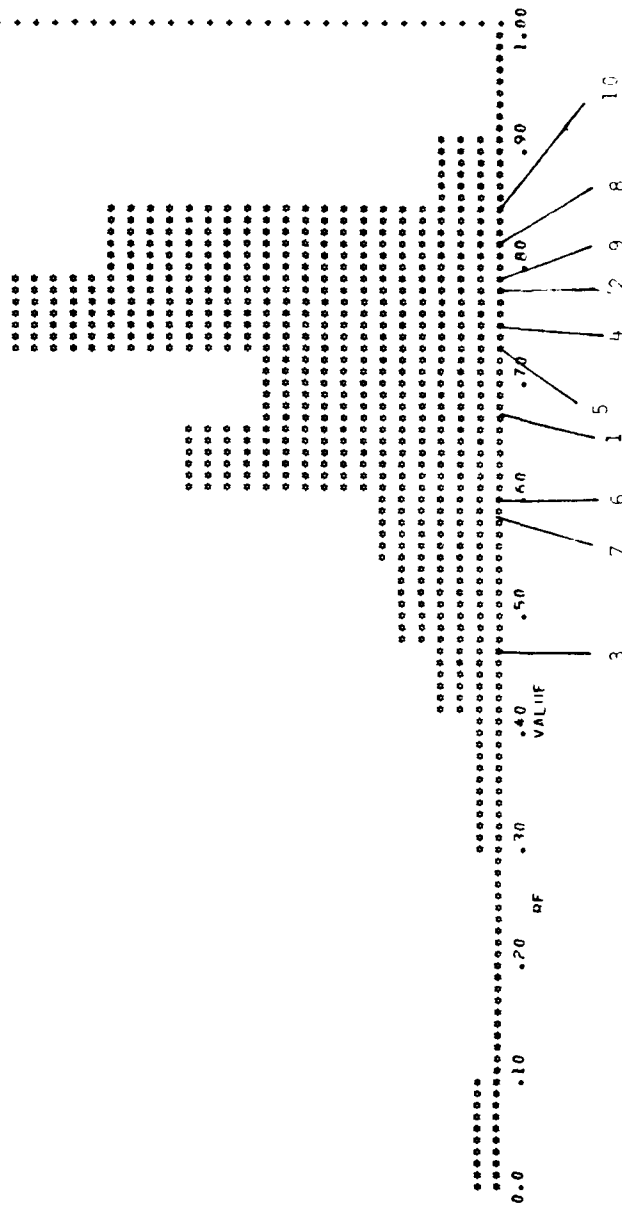


Figure 17-a-I: Oral Treatment, Incubation with Water, Solvent I.

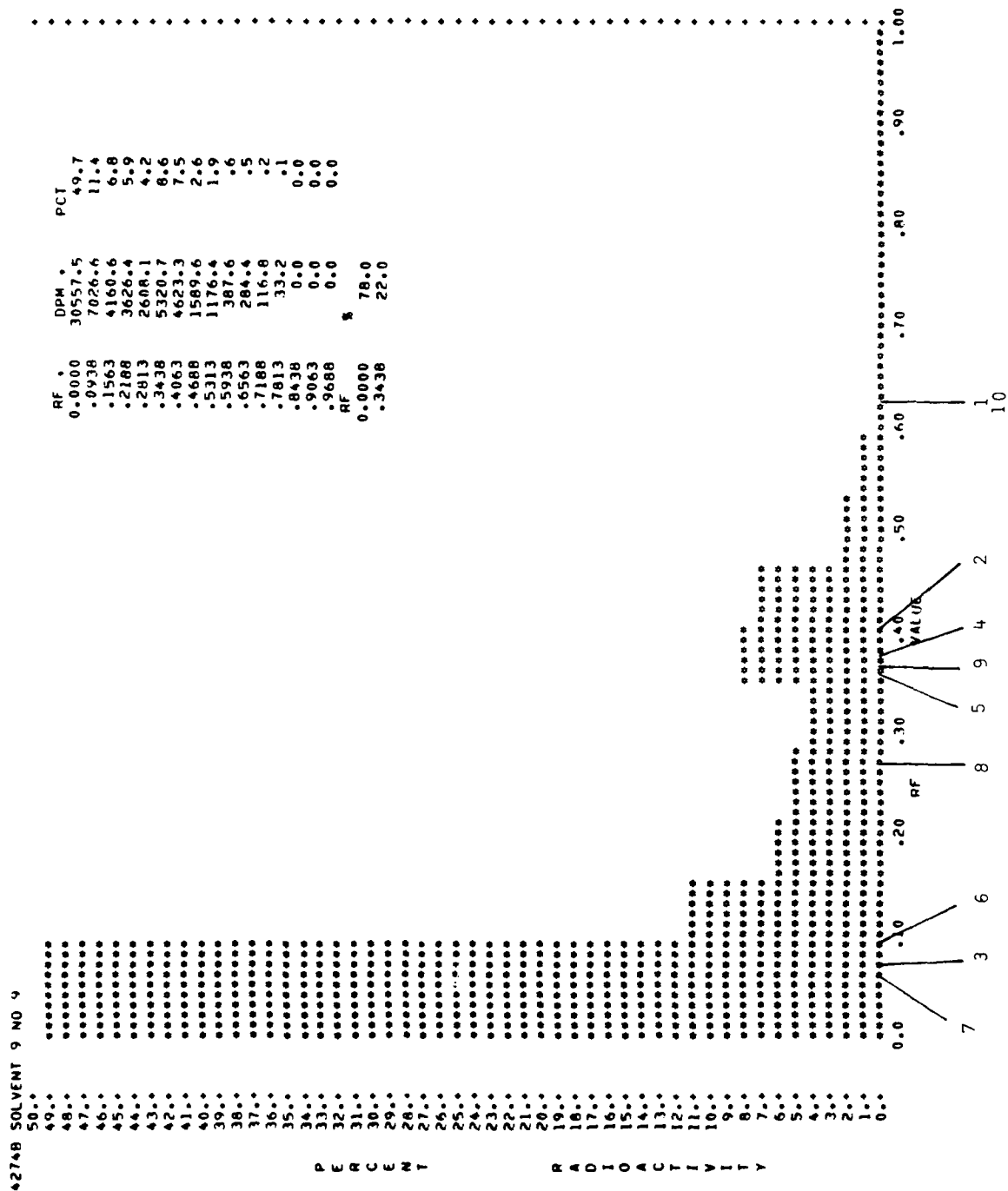


Figure 17-a-IX: Oral Treatment, Incubation with Water, Solvent IX.

42748 SOLVENT 1 NO 10

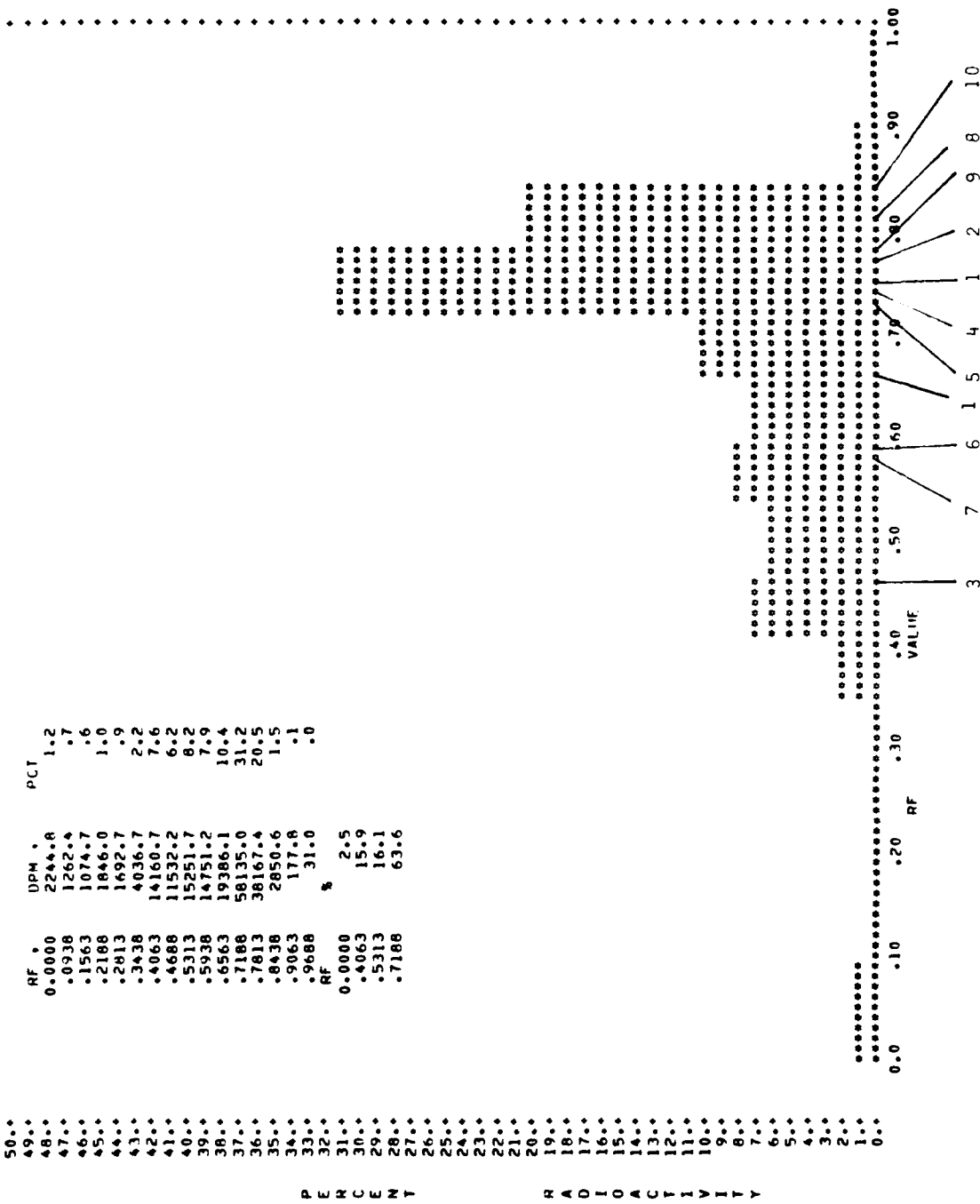


Figure 17-b-I: Oral Treatment, Incubation with B-glucuronidase, Solvent I.

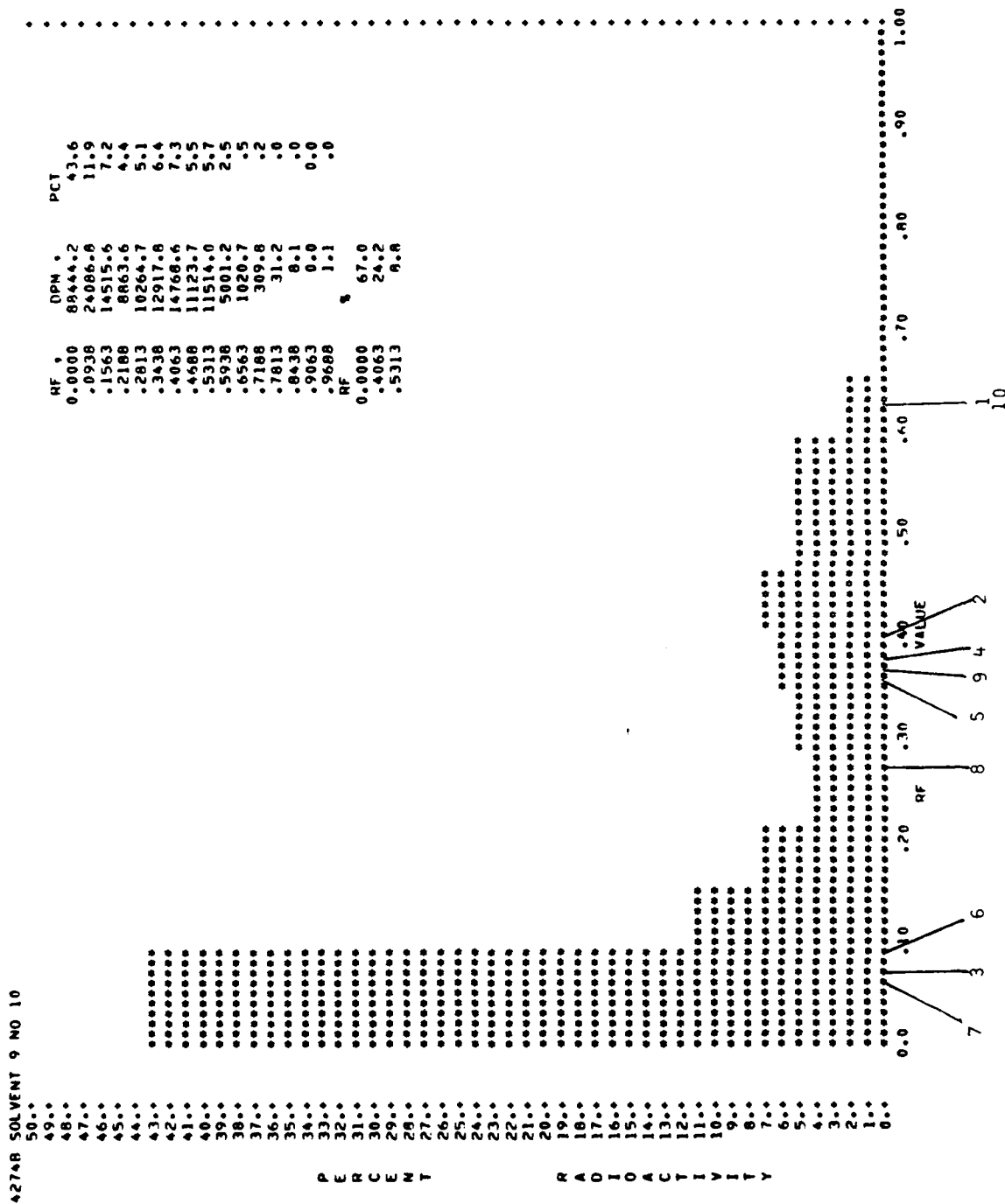


Figure 17-b-IX: Oral Treatment, Incubation with B-glucuronidase, Solvent IX.

42748 SOLVENT 1 NO 5

RF	DPM	PCT
0.0000	342.5	1.0
.0938	199.1	.6
.1563	221.3	.7
.2188	322.5	1.0
.2813	399.1	1.2
.3438	622.0	1.8
.4063	2705.3	8.0
.4688	3014.9	8.9
.5313	3944.8	11.6
.5938	3271.3	9.6
.6563	3944.5	11.6
.7188	6114.2	18.0
.7813	5561.8	16.4
.8438	2930.2	8.6
.9063	316.1	.9
.9688	35.8	.1
RF	%	
.5313	42.7	
.7188	55.7	

P E R R C E N T

R A D I O A C T I V I T Y

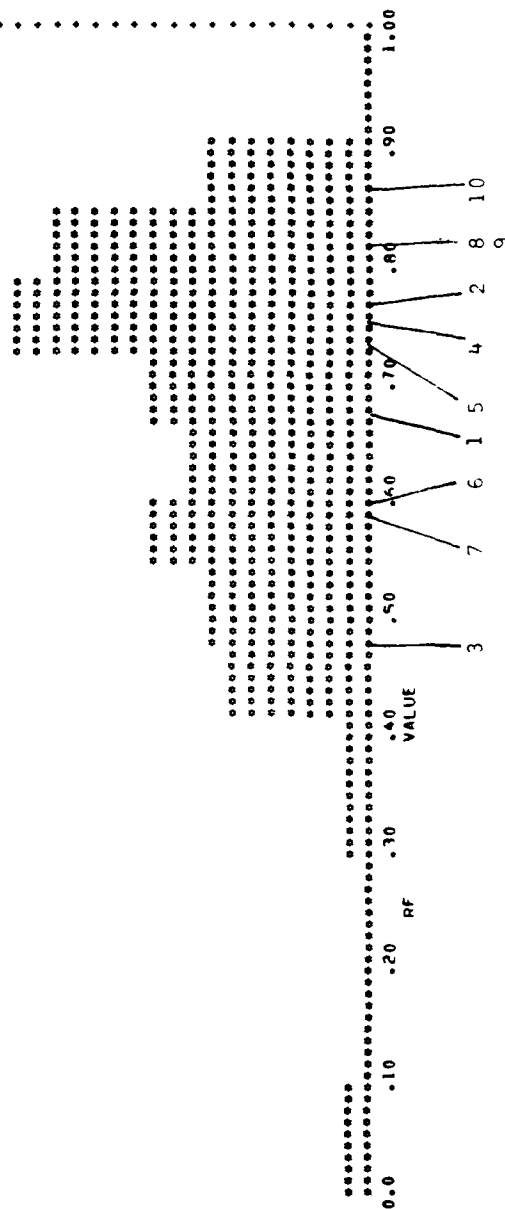


Figure 17-c-I: Dermal Application, Incubation with Water, Solvent I.

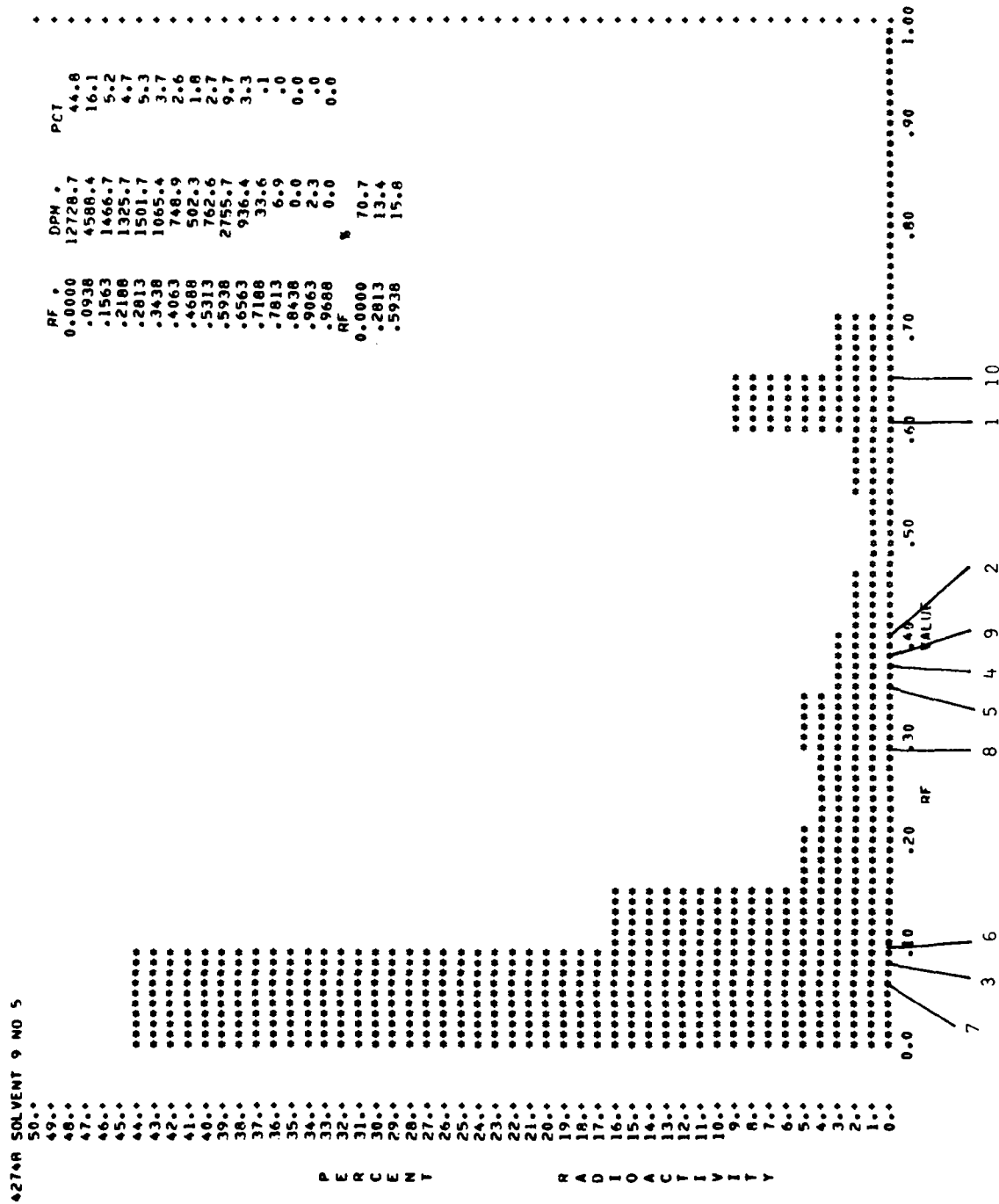


Figure 17-c-IX: Dermal Application, Incubation with Water, Solvent IX.

50.0	RF	DPH	PCT
49.0	0.0000	377.0	.8
48.0	.0936	243.7	.5
47.0	.1563	251.1	.5
46.0	.2188	369.9	.8
45.0	.2813	469.9	1.0
44.0	.3438	698.9	1.5
43.0	.4063	3737.6	8.0
42.0	.4688	3624.3	8.2
41.0	.5313	4905.7	10.5
40.0	.5938	4289.7	9.2
39.0	.6563	4508.7	9.7
38.0	.7188	8630.8	18.5
37.0	.7813	10606.9	22.8
36.0	.8438	3393.1	7.3
35.0	.9063	250.9	.5
34.0	.9688	54.4	.1
33.0	RF		
32.0	.5313	39.8	
31.0	.7813	58.9	

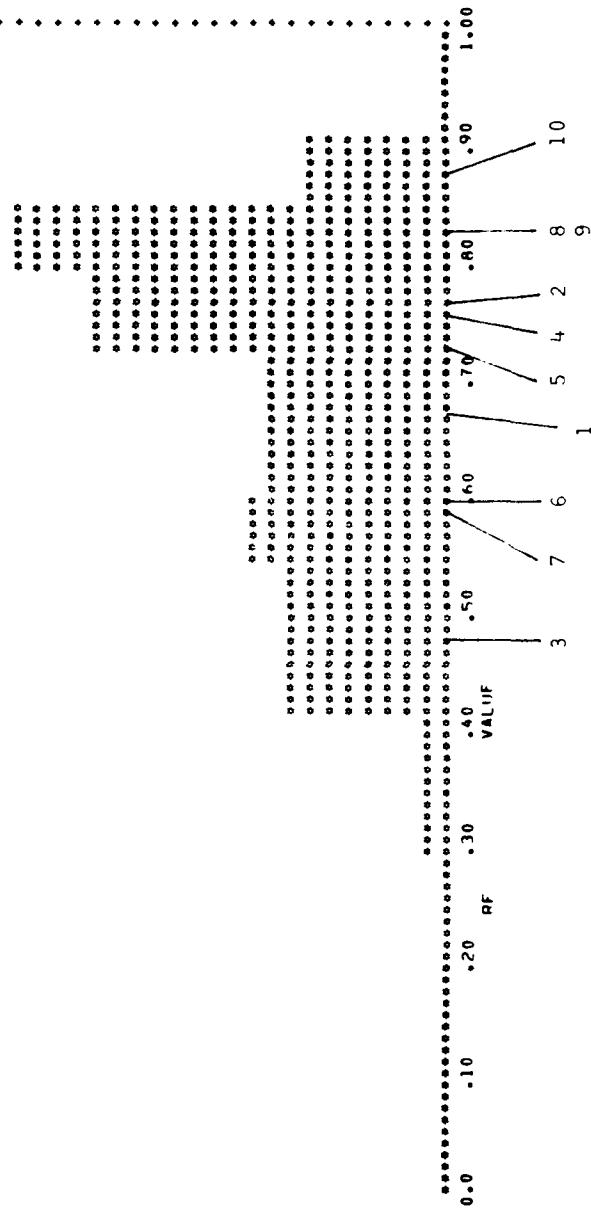


Figure 17-d-I: Dermal Application, Incubation with B-glucuronidase, Solvent I.

42748 SOLVENT 9 NO 6

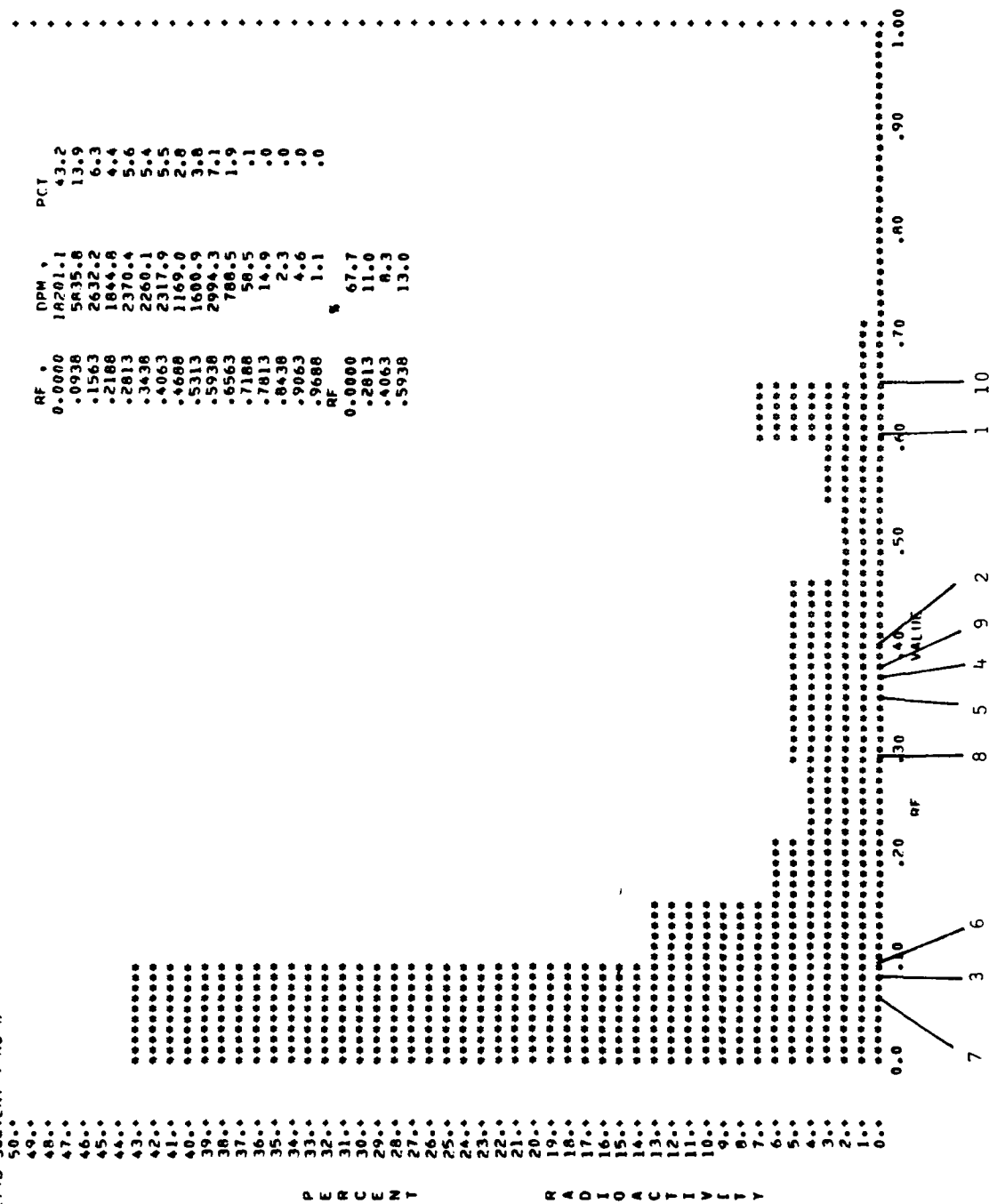


Figure 17-d-IX: Dermal Application, Incubation with B-glucuronidase, Solvent IX.

42748 SOLV 1 NO 5 JAN 27

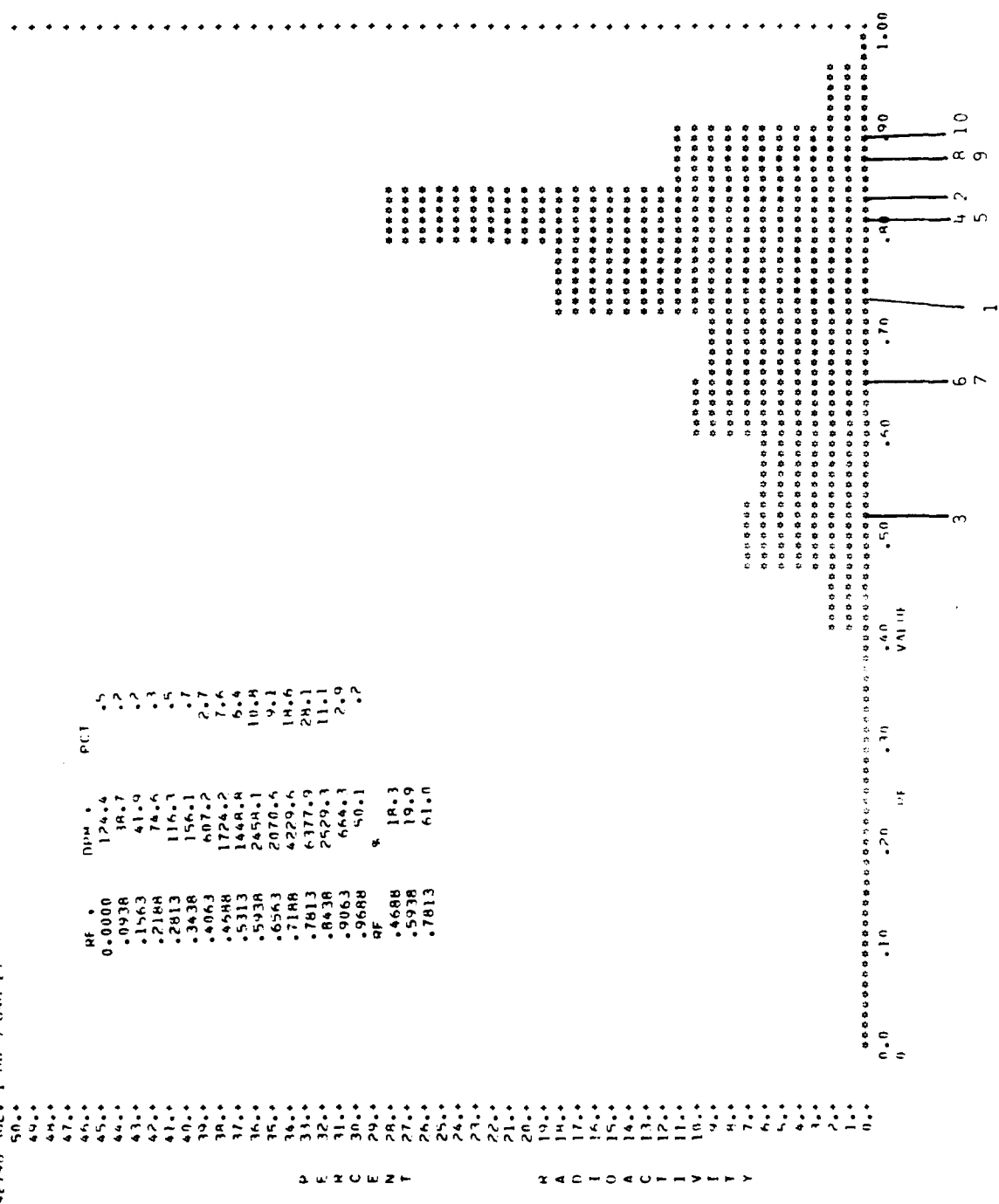


Figure 17-e-1: Oral Treatment, Incubation with Water, Solvent I.

4276H SOLV 4 NO 5

50..	0.0000	6625.9	29.8
49..	.0938	3656.2	16.5
48..	.1563	1824.6	8.2
47..	.2188	1756.4	7.9
46..	.2813	1240.1	5.6
45..	.3438	1202.8	5.4
44..	.4063	1727.3	7.8
43..	.4688	763.3	3.4
42..	.5313	647.9	2.9
41..	.5938	1906.8	8.5
40..	.6563	684.1	3.1
39..	.7188	110.3	.5
38..	.7813	36.1	.2
37..	.8438	16.3	.1
36..	.9063	10.5	.0
35..	.9688	0.0	0.0
34..	RF	%	
33..	0.0000	73.4	
32..	.4063	16.1	
31..	.5938	12.4	

P E R C E N T

W A D I T I V I T Y

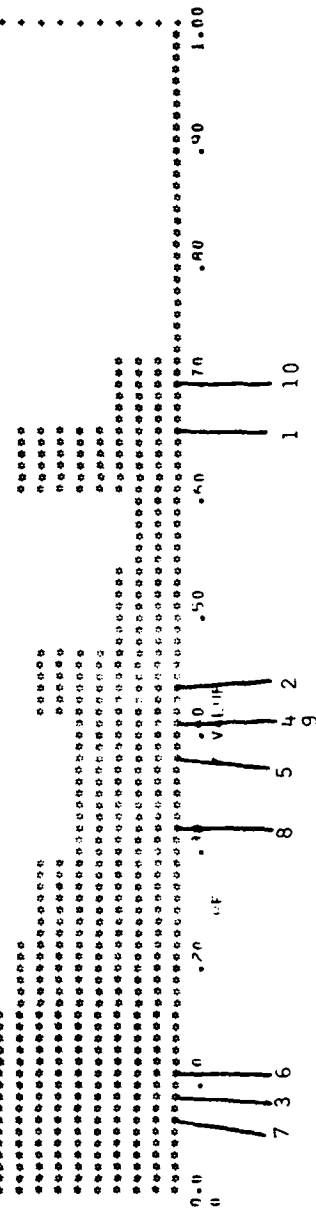


Figure 17-e-IX: Oral Treatment, Incubation with Water, Solvent IX.

42744 SOLV INO6 JAN 27

50.00
43.00
44.00
47.00
46.00
45.00
44.00
43.00
42.00
41.00
40.00
39.00
38.00
37.00
36.00
35.00
34.00
33.00
32.00
31.00
30.00
29.00
28.00
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26.00
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17.00
16.00
15.00
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13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00

P E H C E N T

RF .00000
0.0000
133.7
74.4
74.4
95.3
151.5
314.7
1629.4
3063.6
2013.9
2057.1
1726.5
2794.0
8098.6
7947.2
1326.3
7.0
RF
4688
5938
7813

PLI .4
.2
.2
.3
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4.5
4.9
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25.2
4.2
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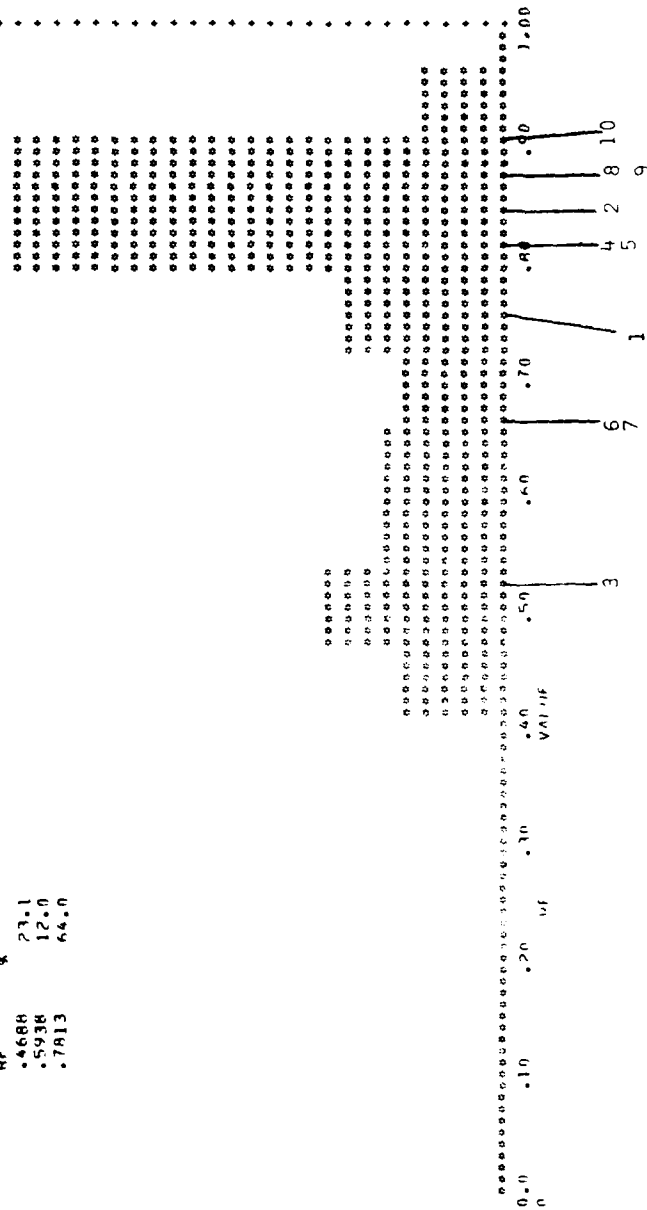


Figure 17-f-l: Oral Treatment, Incubation with B-glucuronidase, Solvent I.

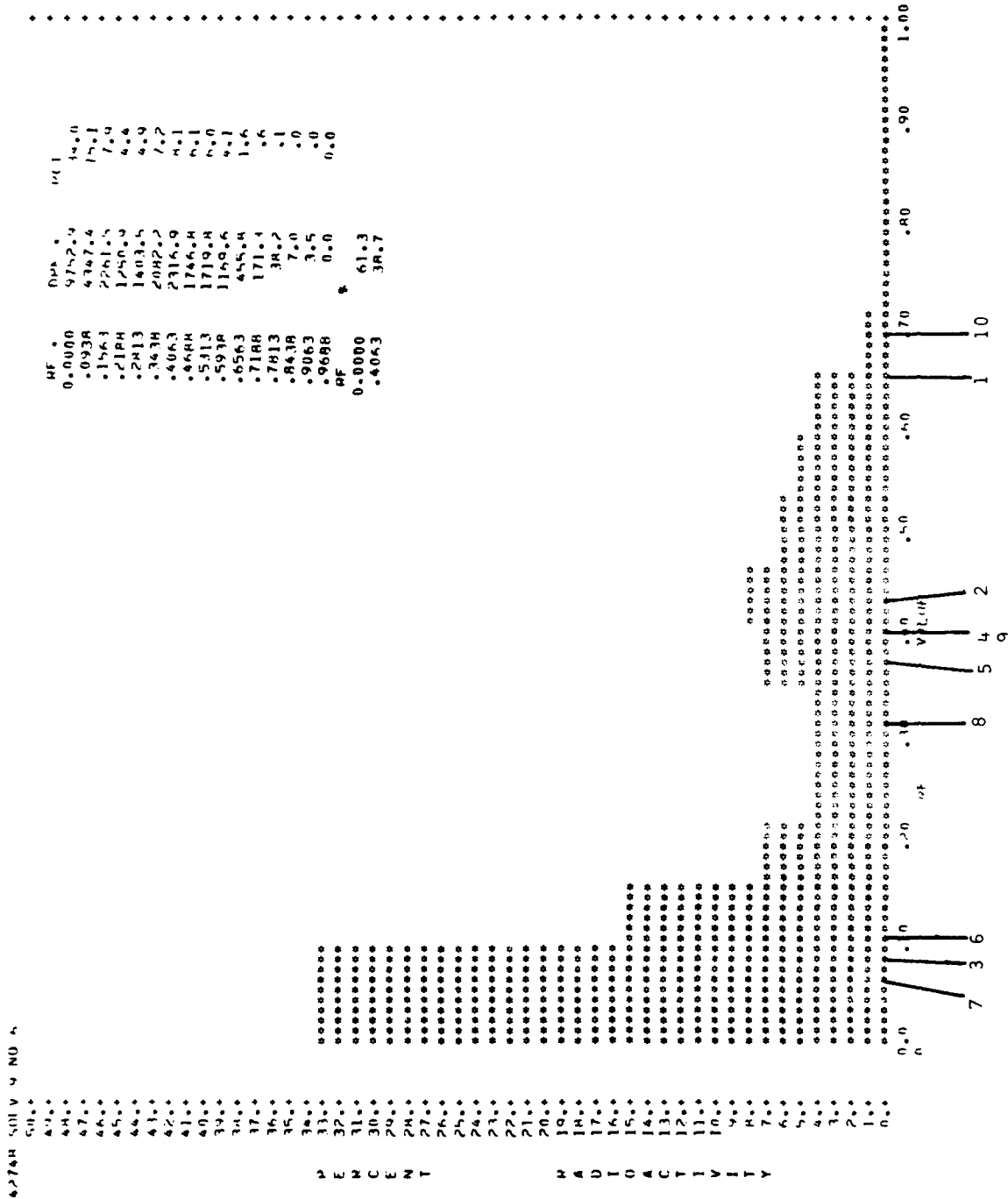


Figure 17-f-IX: Oral Treatment, Incubation with B-glucuronidase, Solvent IX.

4274R SOLV 1 NO 11

RF	DPM	PCT
0.0000	19.4	.4
.0938	7.0	.2
.1563	14.1	.4
.2188	10.5	.3
.2813	42.0	1.2
.3438	100.9	3.0
.4063	266.9	7.9
.4688	276.8	8.2
.5313	373.3	11.1
.5938	586.9	17.4
.6563	952.4	28.3
.7188	636.0	18.9
.7813	74.4	2.2
.8438	3.5	.1
.9063	2.3	.1
.9688	0.0	0.0
RF	98.1	

P E R C E N T

R A U T I D A C T I V I T Y

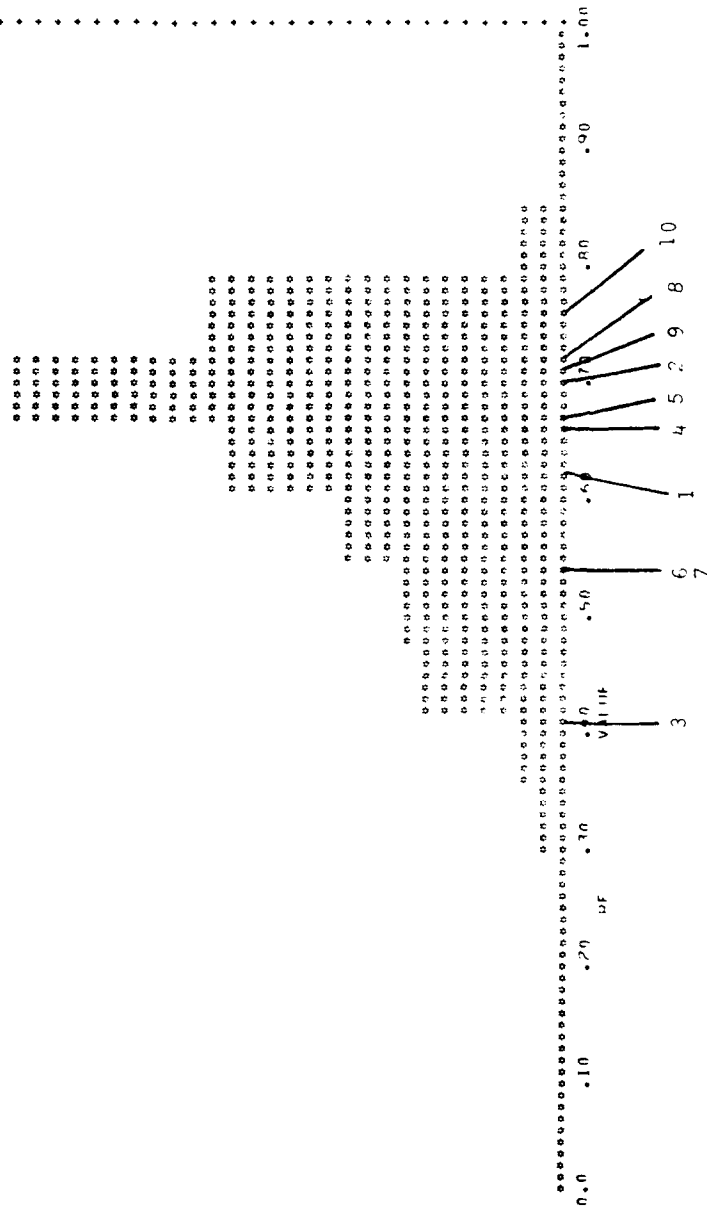


Figure 17-g-I: Dermal Application, Incubation with Water, Solvent I.

4274H SOLV 9 NO 11

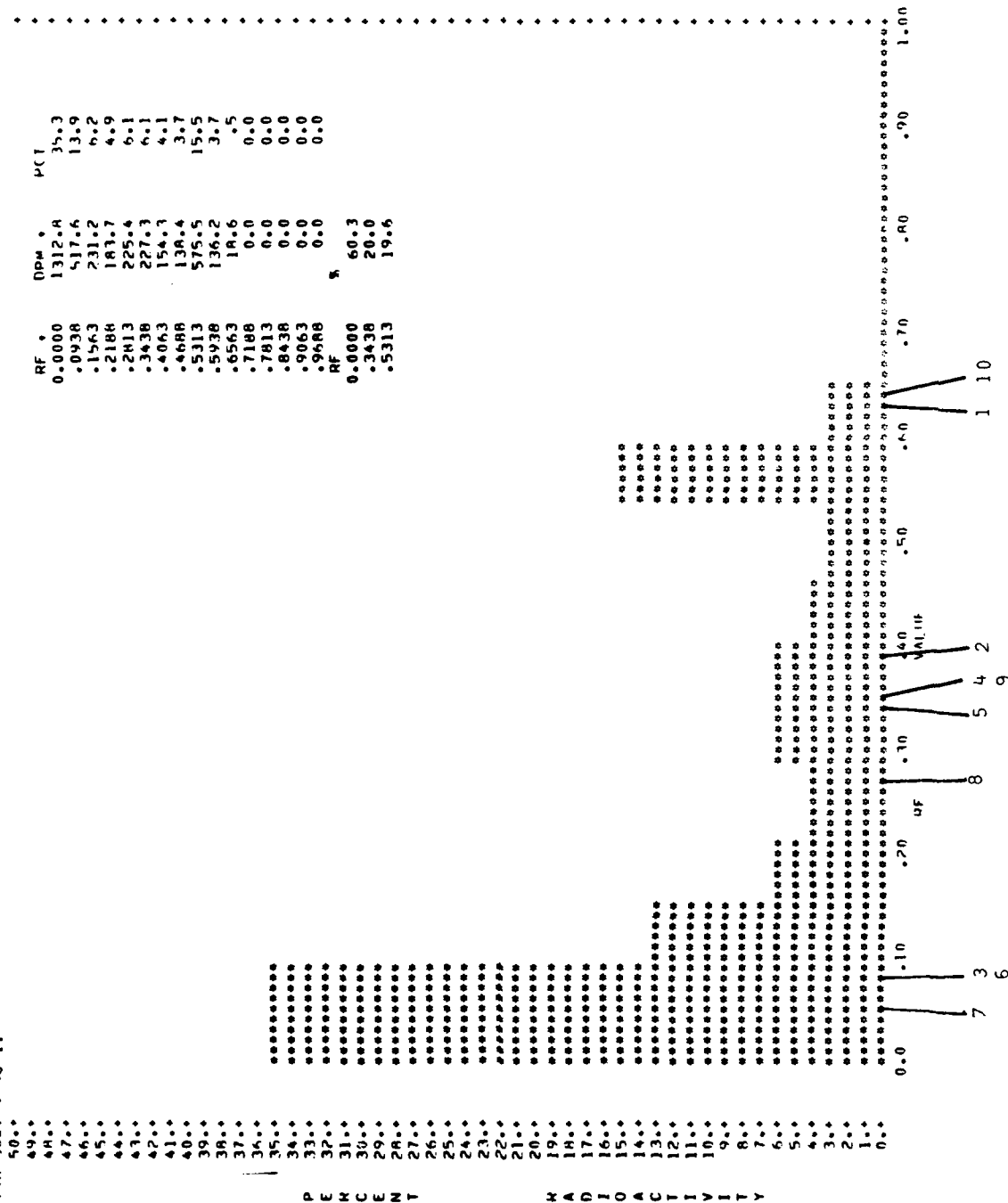


Figure 17-g-IX: Dermal Application, Incubation with Water, Solvent IX.

4274R SOLV 1 NO 12

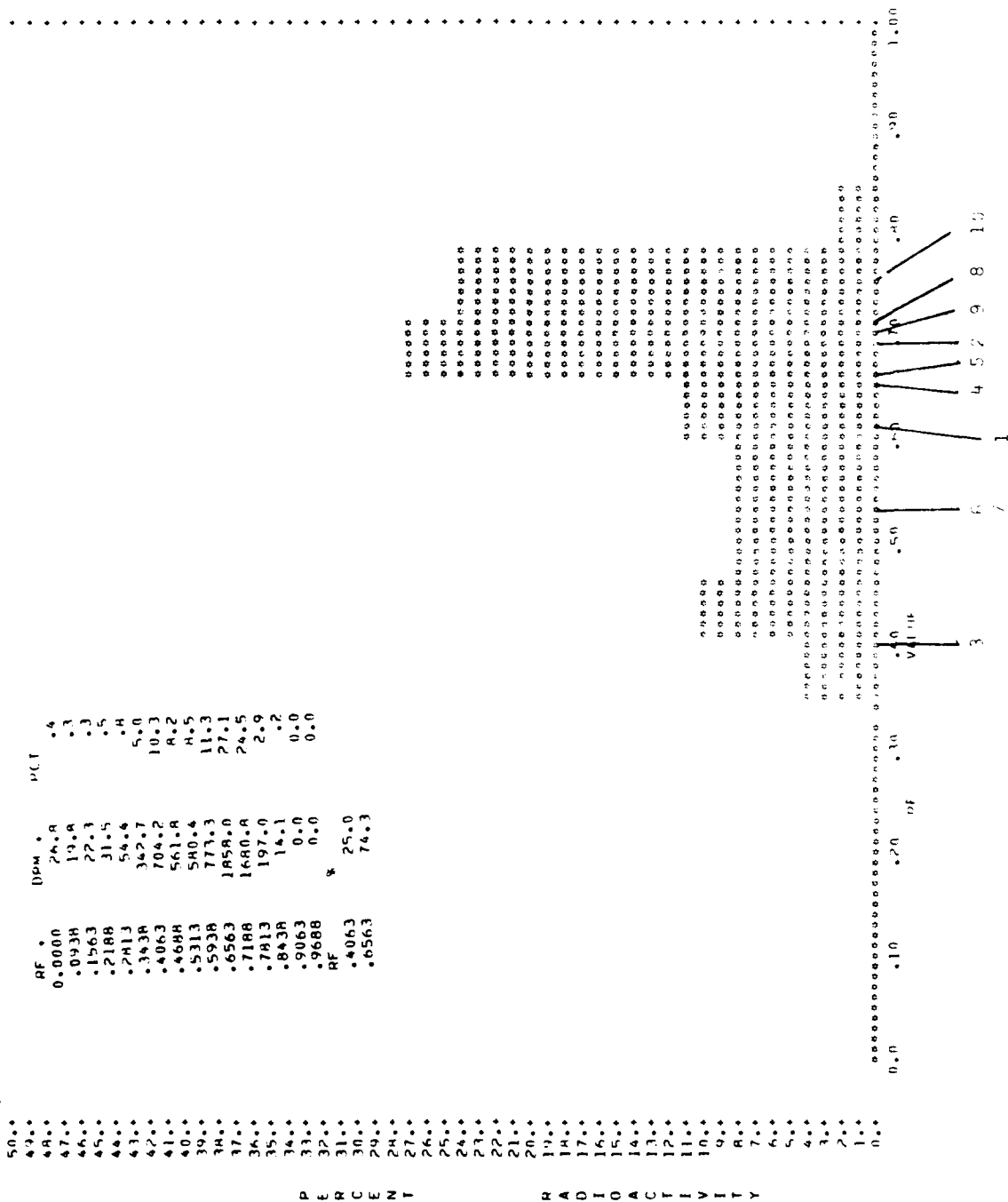


Figure 17-1-1: Bermal Application, Incubation with B-glucuronidase, Solvent 1.

4274H SOLV 9 NO 12

50.0	RF	0.0000	DPM	2470.7	PCT	37.6
49.0		.0438		1079.1		16.4
48.0		.1563		384.9		5.9
47.0		.2148		282.4		4.3
46.0		.2813		442.5		6.7
45.0		.3438		525.8		8.0
44.0		.4063		479.0		7.3
43.0		.4688		287.4		4.4
42.0		.5313		455.8		6.9
41.0		.5938		120.9		1.8
40.0		.6563		47.8		.7
39.0		.7188		1.2		.0
38.0		.7813		0.0		0.0
37.0		.8438		0.0		0.0
36.0		.9063		0.0		0.0
35.0		.9688		0.0		0.0
34.0		RF				
33.0		0.0000	%	64.1		
32.0		.3438		26.3		
31.0		.5313		9.5		
30.0						
29.0						
28.0						
27.0						
26.0						
25.0						
24.0						
23.0						
22.0						
21.0						
20.0						
19.0						
18.0						
17.0						
16.0						
15.0						
14.0						
13.0						
12.0						
11.0						
10.0						
9.0						
8.0						
7.0						
6.0						
5.0						
4.0						
3.0						
2.0						
1.0						
0.0						

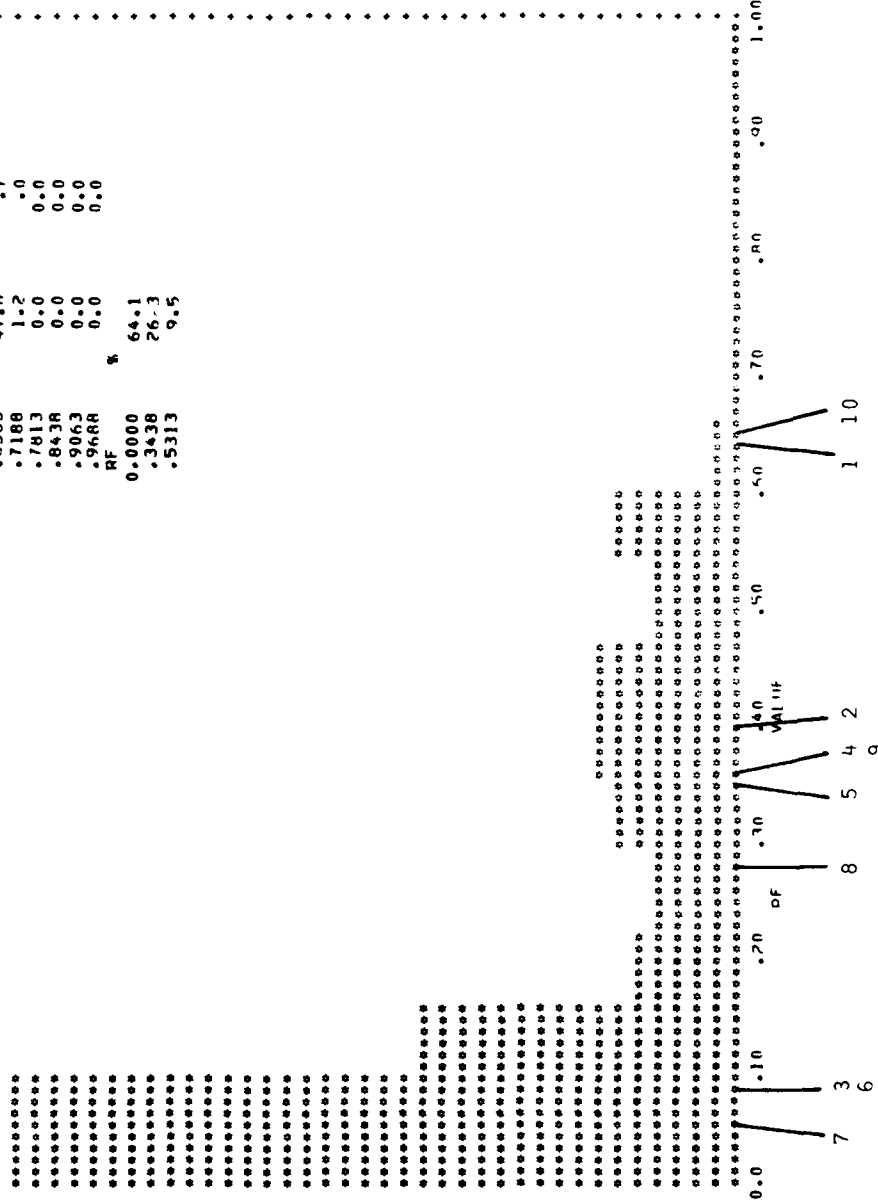


Figure 17-h-IX: Dermal Application, Incubation with β -glucuronidase, Solvent IX.

4274R SOLV I NO 9

RF	PPM	PCT
0.0000	115.0	.6
.0938	14.5	.2
.1563	62.2	.3
.2188	96.1	.5
.2813	158.5	.8
.3438	296.5	1.6
.4063	1227.7	6.6
.4688	1223.1	6.5
.5313	1996.5	10.7
.5938	3336.9	17.9
.6563	5616.2	30.6
.7188	4066.0	21.8
.7813	390.7	2.1
.8438	56.3	.3
.9063	9.4	.1
.9688	0.0	0.0
RF		
.4063	16.4	
.6563	82.8	

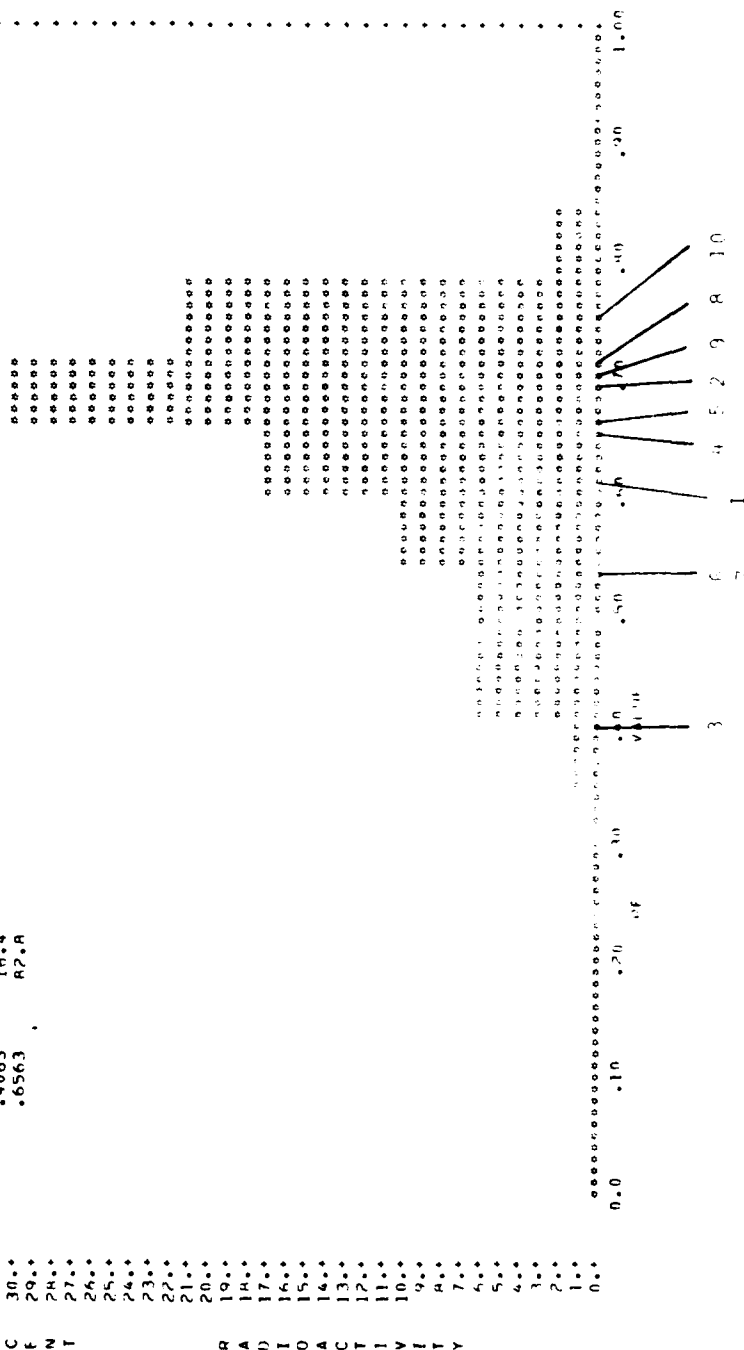


Figure 17-k-1: Oral Treatment, Incubation with Water, Solvent 1.

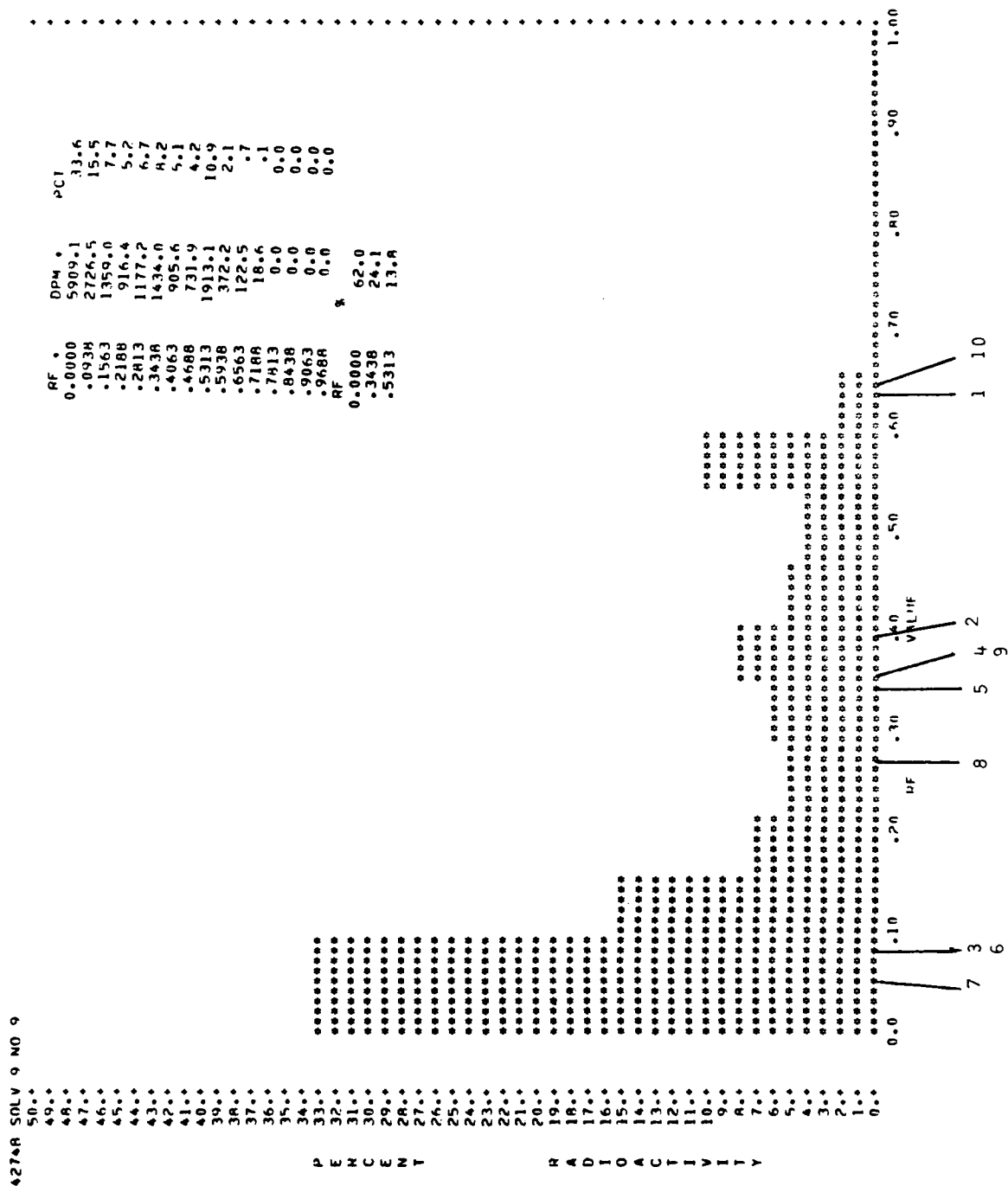


Figure 17-k-IX: Oral Treatment, Incubation with Water, Solvent IX.

427-00 SOLV 1 000 M

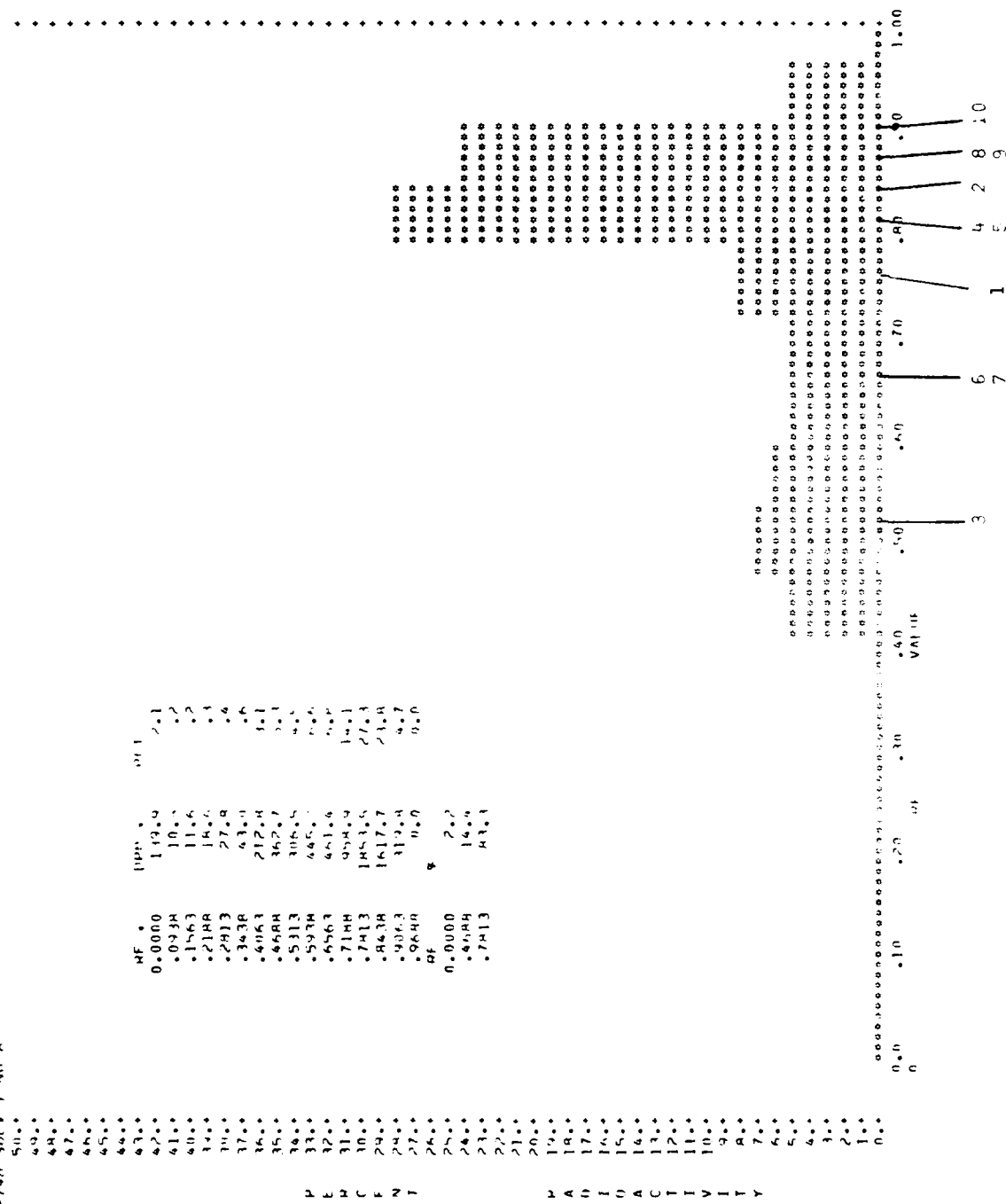


Figure 17-1-I: Dermal Application, Incubation with Water, Solvent I.

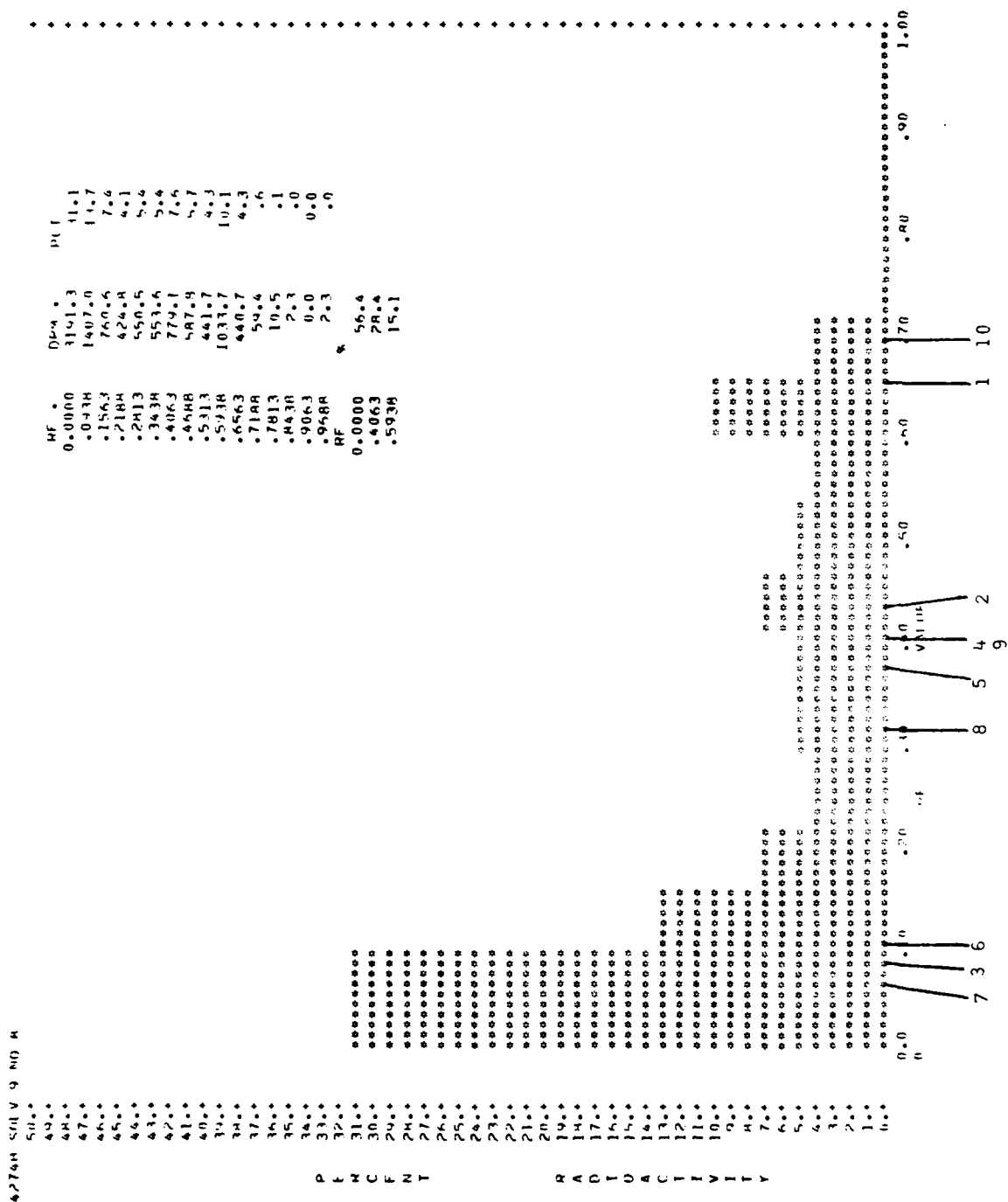


Figure 17-1-IX: Dermal Application, Incubation with Water, Solvent IX.

Figure 18: TLC of Ethyl Acetate-Extractable Products Obtained from 4-Hr Urine of Male Rats Treated Orally or Intratracheally with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 18 follows

42754 SOLV 1 MD 9

WT	100%	101
0.0000	174.6	0.0
.0034	240.1	1.2
.1563	205.4	1.1
.2144	236.2	1.2
.2413	239.6	1.2
.3434	313.7	1.6
.4063	215.5	1.1
.4684	1271.7	6.6
.5313	1959.5	10.1
.5934	174.9	8.4
.6563	3962.2	20.4
.7184	4751.7	24.5
.7813	1493.0	3.7
.8434	406.6	2.1
.9063	44.8	.2
.9684	17.6	.1
RF		
0.0000	4.2	
.4063	21.7	
.5313	19.0	
.7184	55.0	

P F H N C F N T

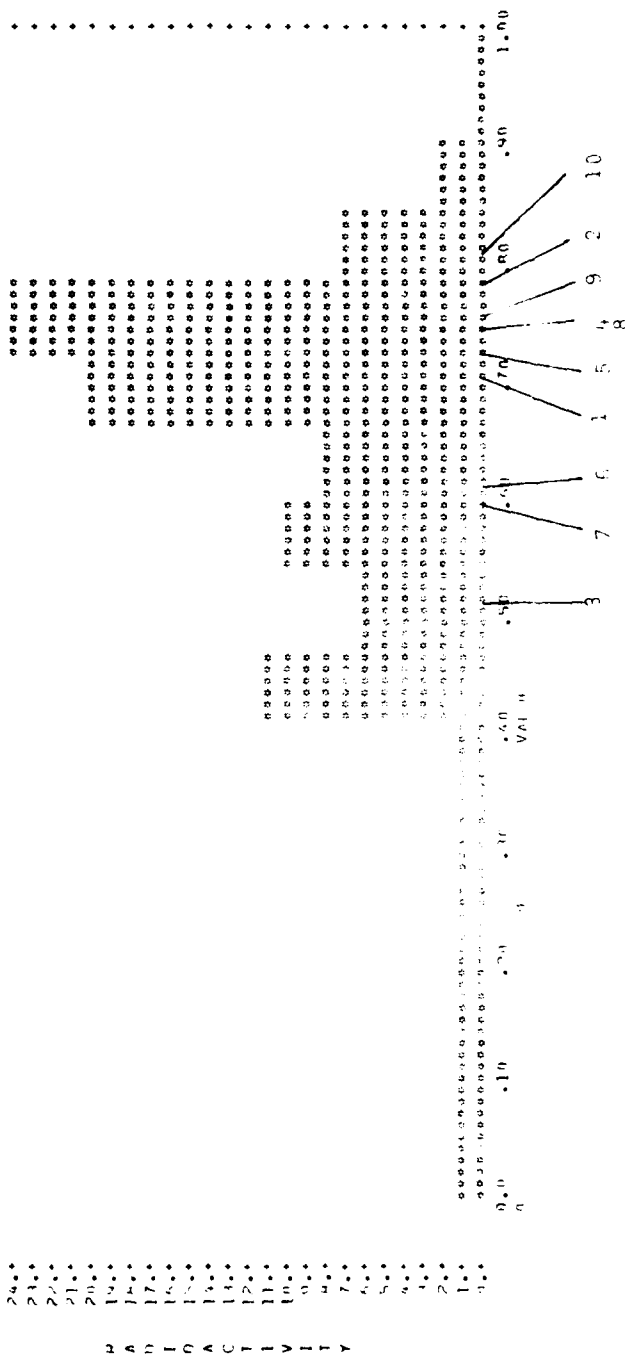


Figure 18-a-I: Oral Treatment, Incubation with Water, Solvent 1

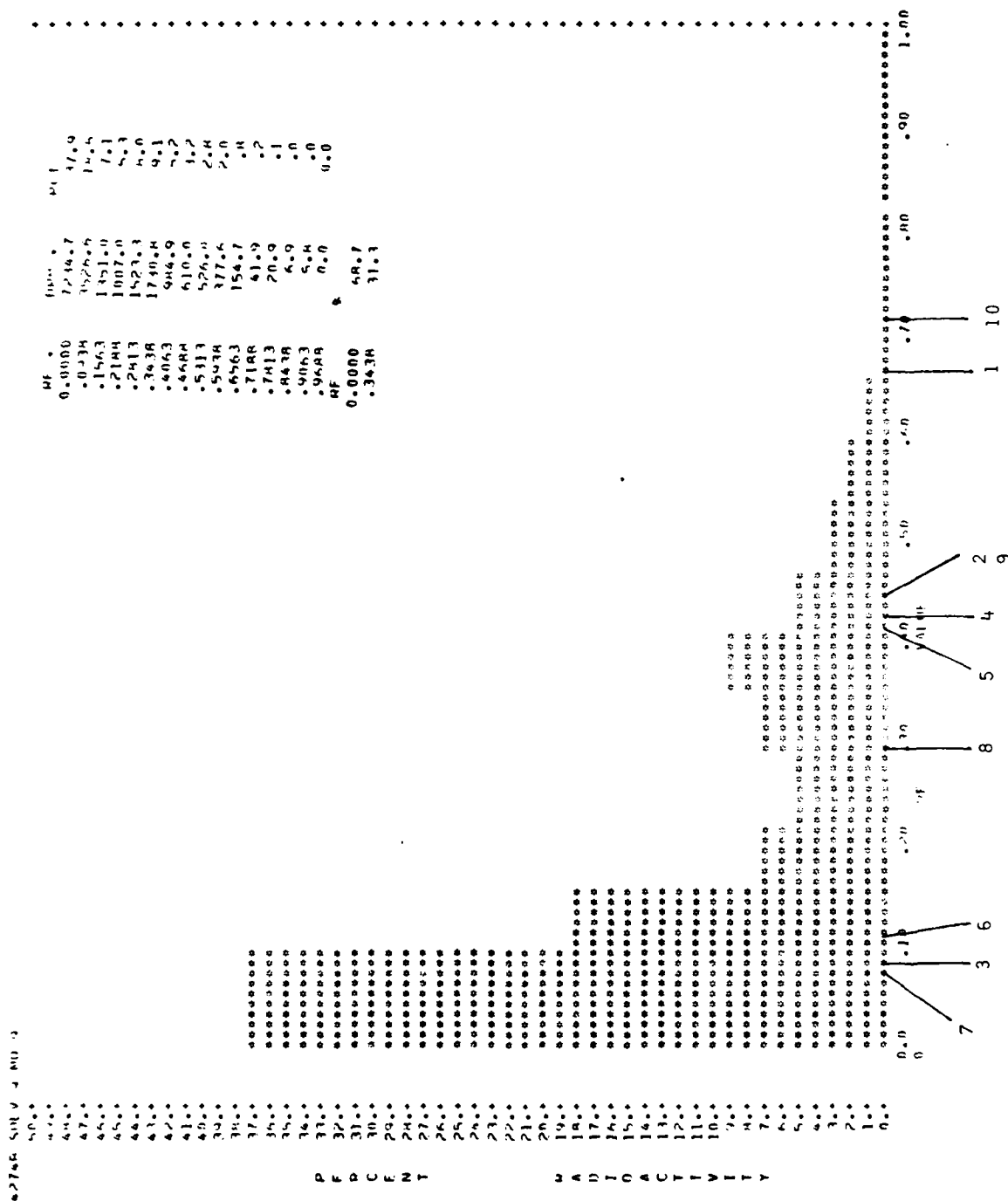


Figure 18-a-IX: Oral Treatment, Incubation with Water, Solvent IX.

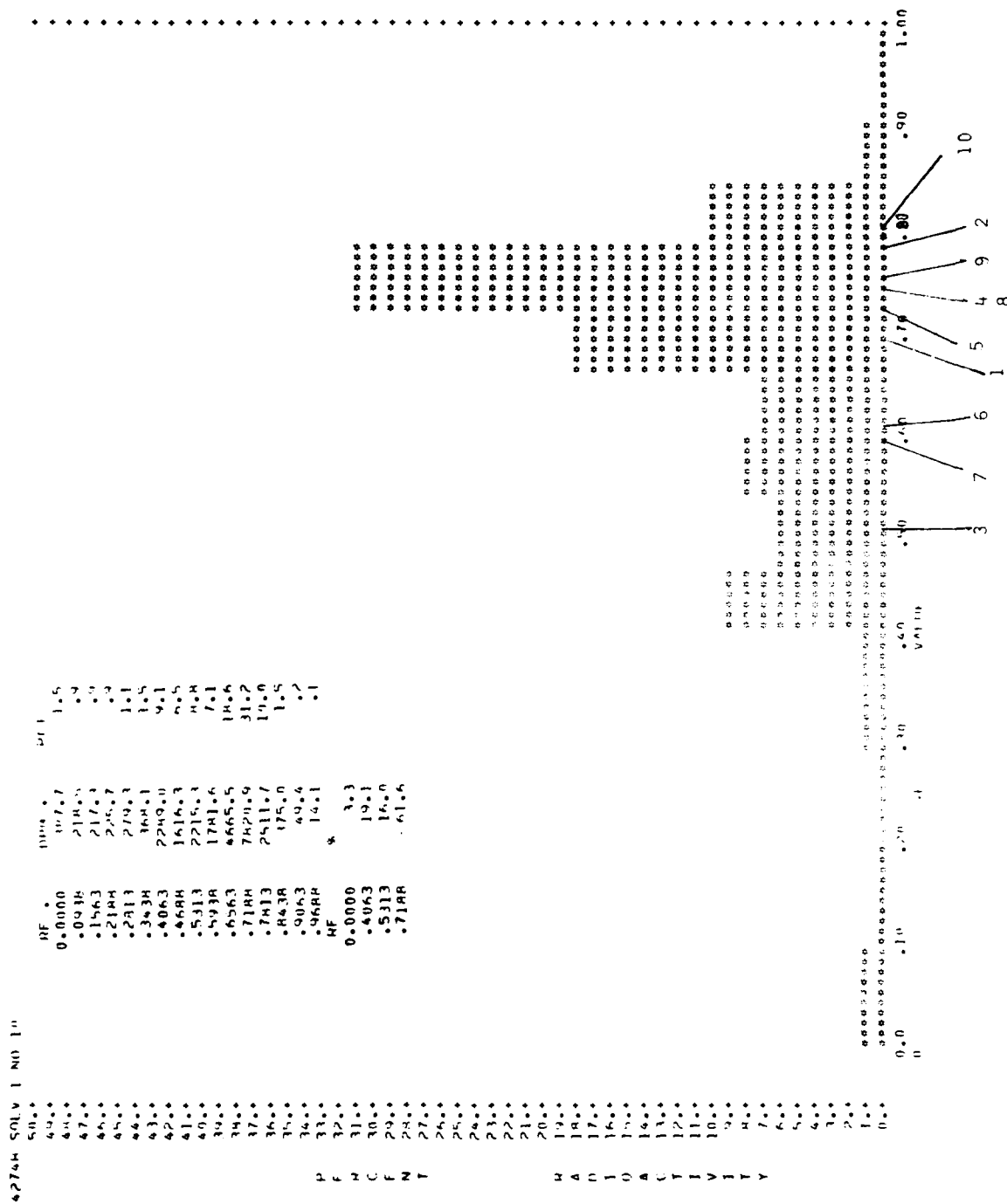


Figure 18-b-I: Oral Treatment, Incubation with B-glucuronidase, Solvent I

4274M SOLV 1 NO 11

50..	RF .	1000 .	PH I
49..	0.0000	204.4	1.7
48..	.0438	112.3	.9
47..	.1563	94.9	.4
46..	.2188	101.7	.8
45..	.2813	124.2	1.0
44..	.3438	172.5	1.4
43..	.4063	466.4	4.6
42..	.4688	797.7	6.5
41..	.5313	1067.6	8.7
40..	.5938	1127.8	9.2
39..	.6563	2189.6	17.8
38..	.7188	4824.9	35.9
37..	.7813	1153.9	9.4
36..	.8438	142.4	1.2
35..	.9063	9.2	.1
34..	.9688	19.7	.2
33..	RF	%	
32..	0.0000	3.3	
31..	.7188	96.6	
30..			
29..			
28..			
27..			
26..			
25..			
24..			
23..			
22..			
21..			
20..			
19..			
18..			
17..			
16..			
15..			
14..			
13..			
12..			
11..			
10..			
9..			
8..			
7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E W C E N T

M A D I O A C T I V

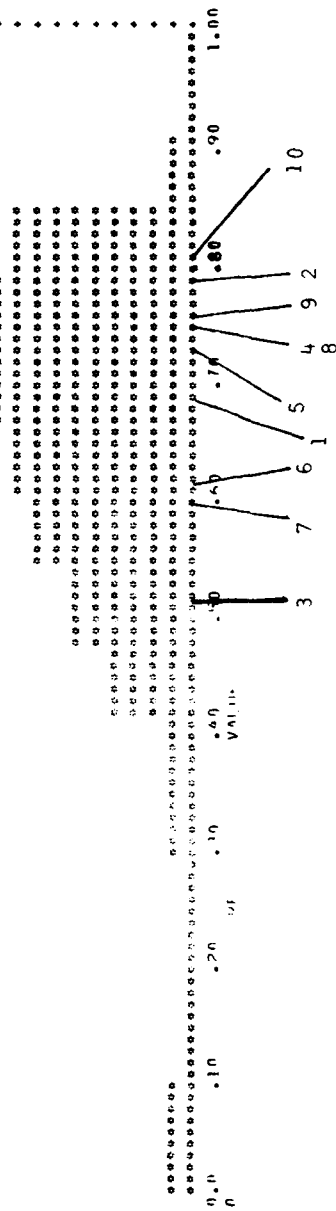


Figure 18-c-I: Intratracheal Instillation, Incubation with Water, Solvent I.

42740 SOL J 3 80 11

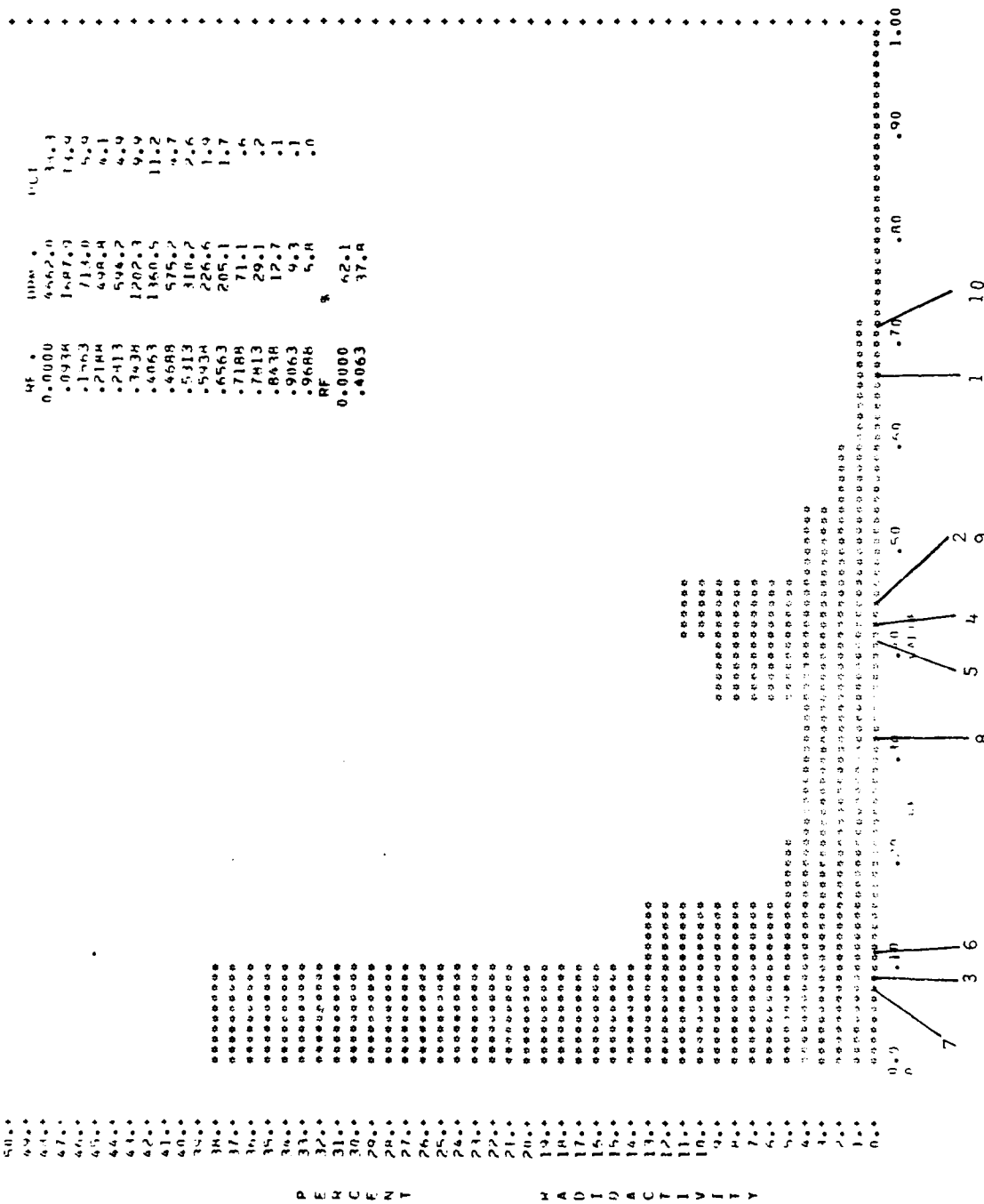


Figure 18-c-IX: Intratracheal Instillation, Incubation with Water, Solvent IX.

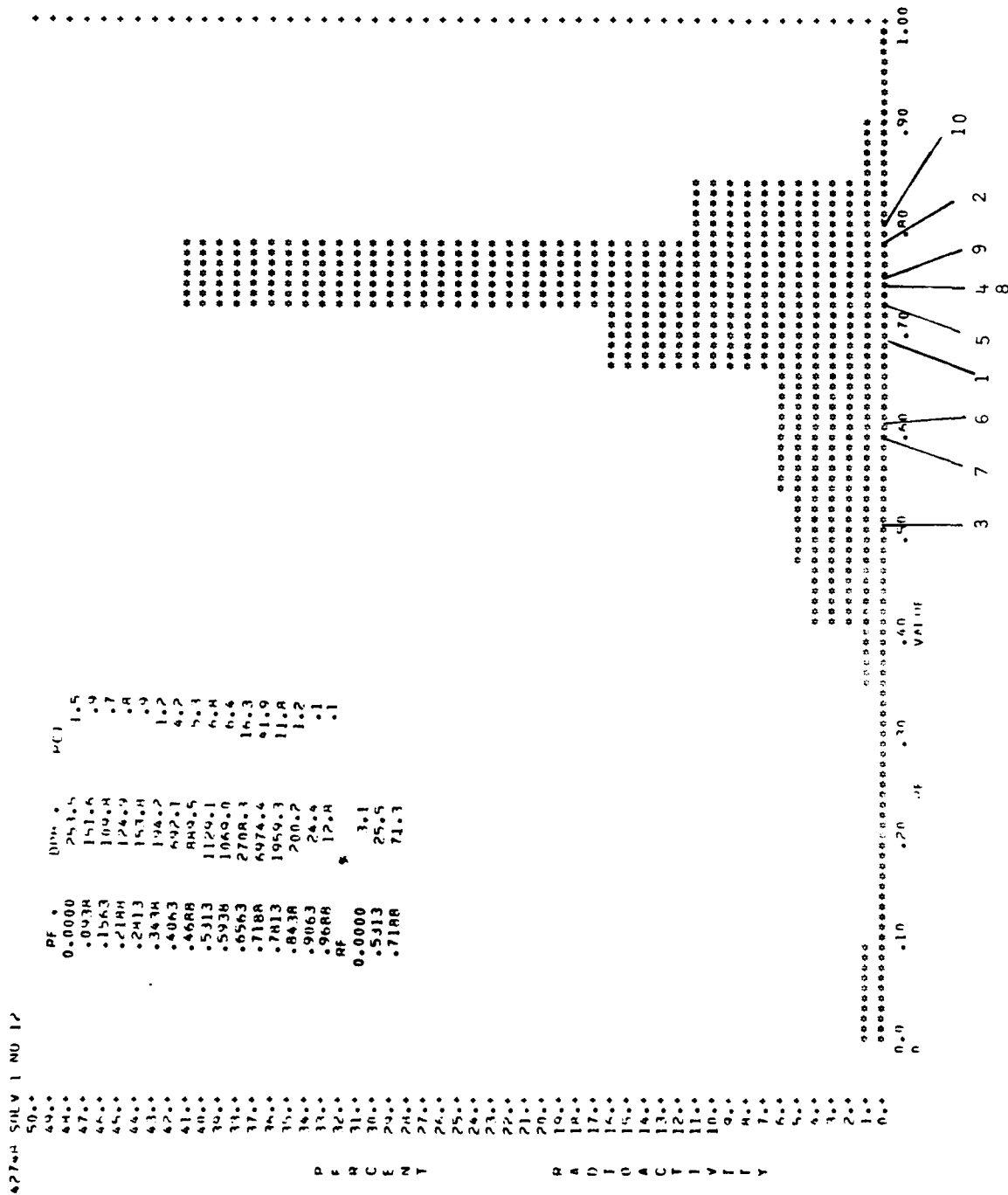


Figure 18-d-I: Intratracheal Instillation, Incubation with B-glucuronidase, Solvent I.

Figure 19: TLC of Ethyl Acetate-Extractable Products Obtained from 4-Hr Urine of Female Rats Treated Orally or Intratracheally with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 19 follows

42754 536V 1 NO 13

50.0	HF	DDM	DCI
49.0	0.0000	271.0	1.2
48.0	.0438	157.2	1.9
47.0	.1563	110.7	1.3
46.0	.2184	100.0	1.2
45.0	.2813	123.5	1.5
44.0	.3438	168.6	2.0
43.0	.4063	1276.2	15.1
42.0	.4688	1031.7	12.2
41.0	.5313	454.2	5.6
40.0	.5938	519.7	6.2
39.0	.6563	800.5	9.5
38.0	.7188	1287.2	15.2
37.0	.7813	1805.7	21.4
36.0	.8438	277.4	1.3
35.0	.9063	57.5	.7
34.0	.9688	1.2	.0
33.0	RF		
32.0	0.0000	7.6	
31.0	.4063	36.2	
30.0	.7813	56.2	

P E M C F N Y

H A U I O A C F I V I Y

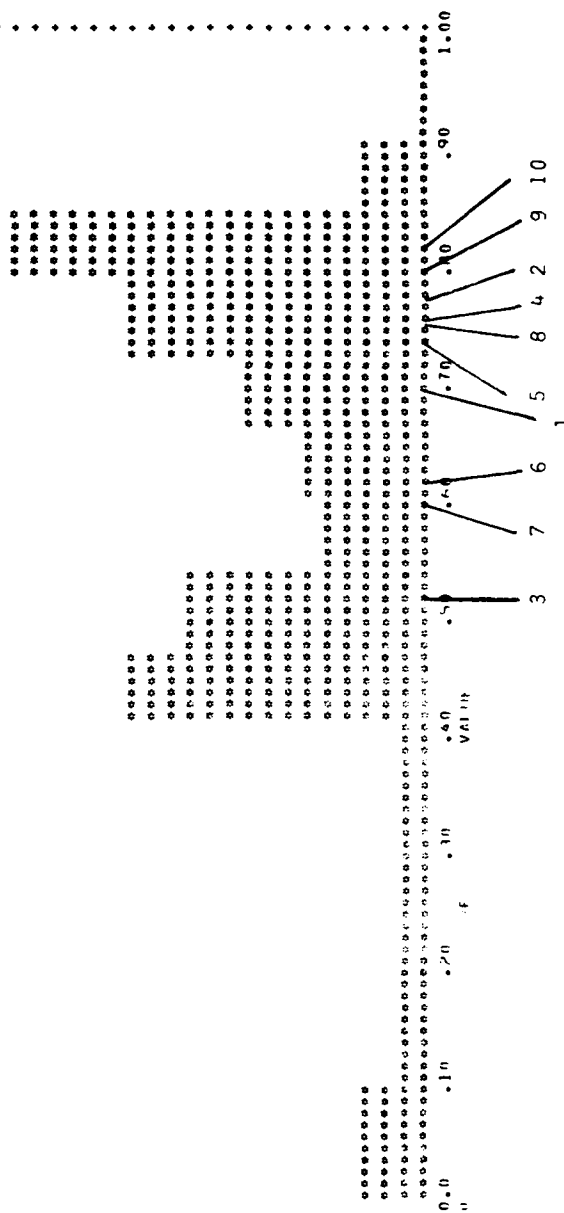


Figure 19-a-I: Oral Treatment, Incubation with Water, Solvent I

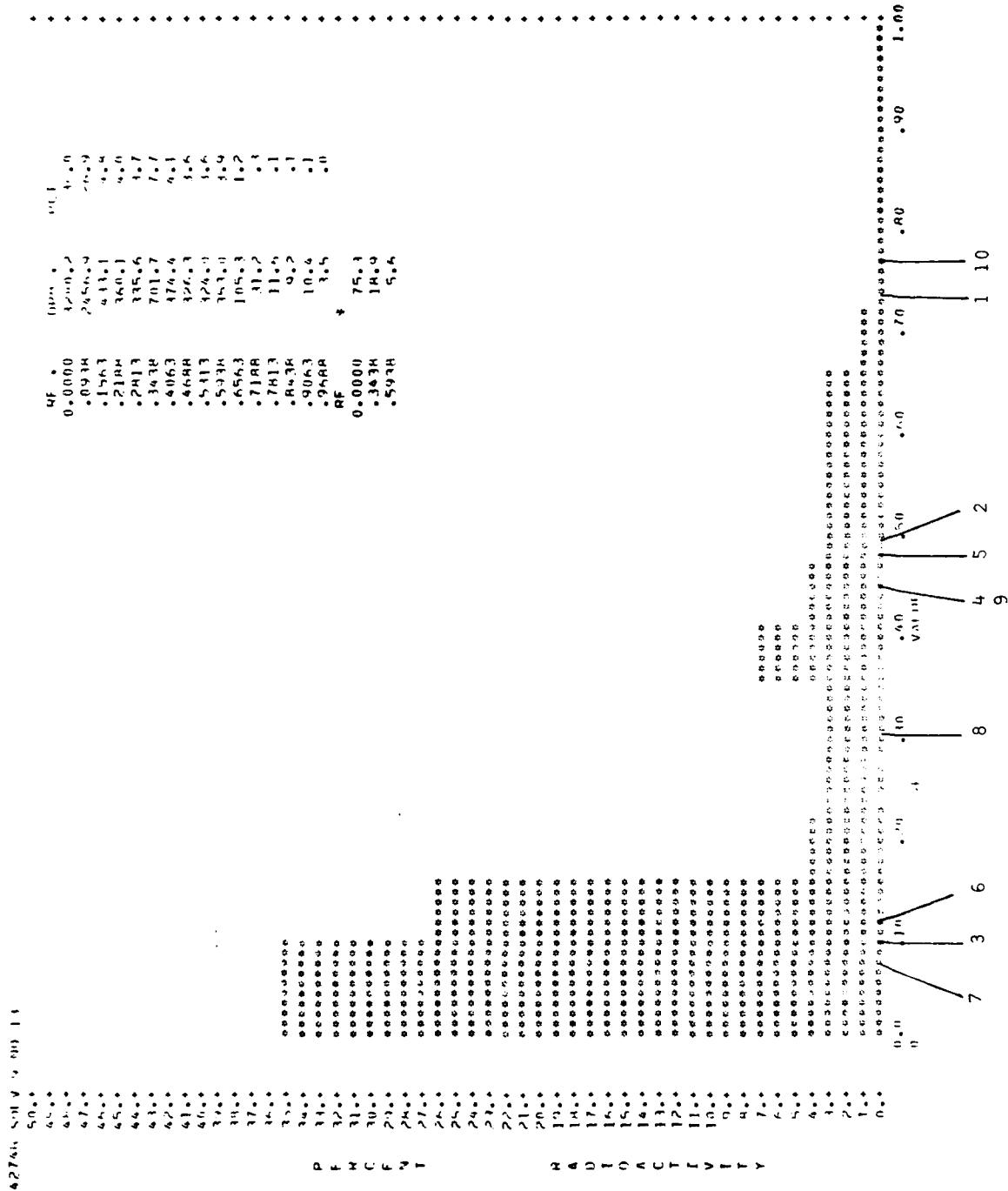


Figure 19-a-IX: Oral Treatment, Incubation with Water, Solvent IX

42744 SOLV I NO 14

PF	DPH	PL
0.0000	348.0	1.7
.0914	201.2	1.0
.1563	154.9	.8
.2184	106.2	1.1
.2813	213.0	1.5
.3434	310.2	1.8
.4063	3172.5	11.0
.4684	2204.0	4.2
.5313	848.4	6.4
.5938	1278.6	10.5
.6563	2108.7	22.7
.7188	4545.5	19.0
.7813	3818.6	1.0
.8438	600.0	.4
.9063	77.9	.0
.9684	8.2	
PF		
0.0000	1.5	
.4063	14.4	
.7188	62.0	

P E R C E N T

H A D T O A C T I V I T Y

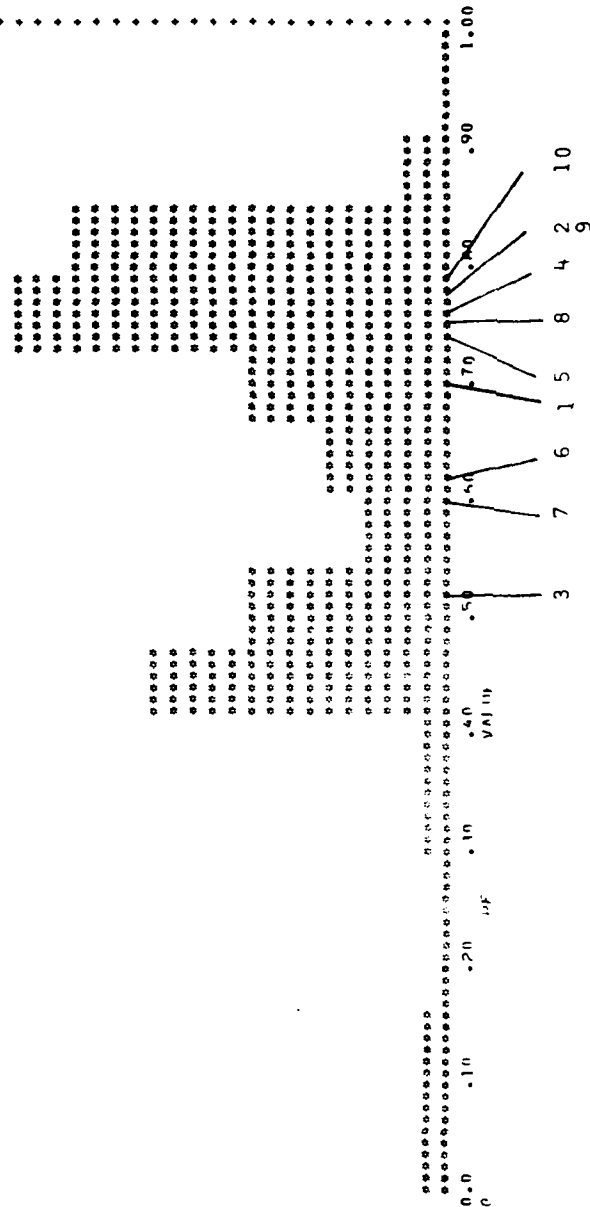


Figure 19-b-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

62744 NIB 9 10 14

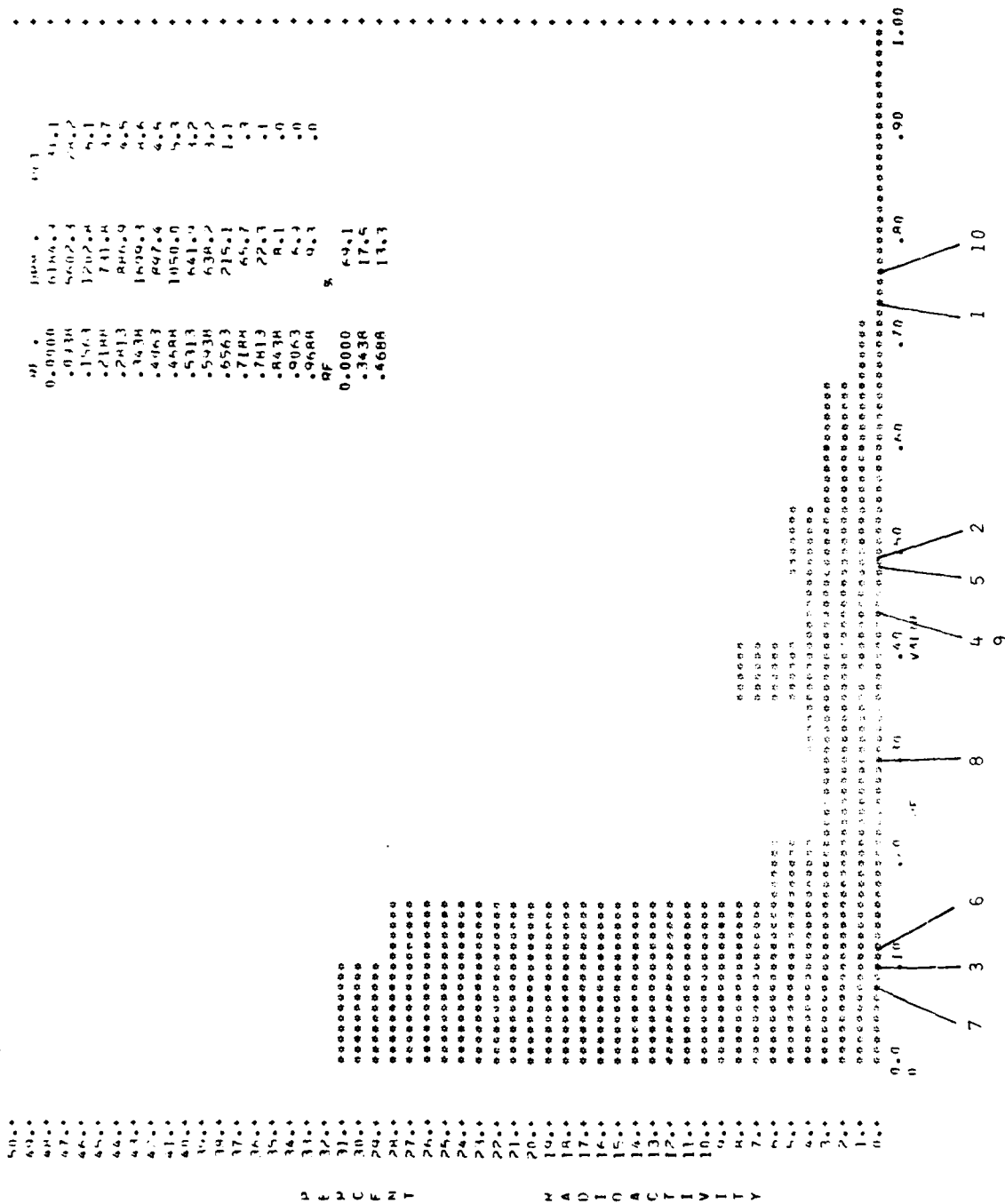


Figure 19-b-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

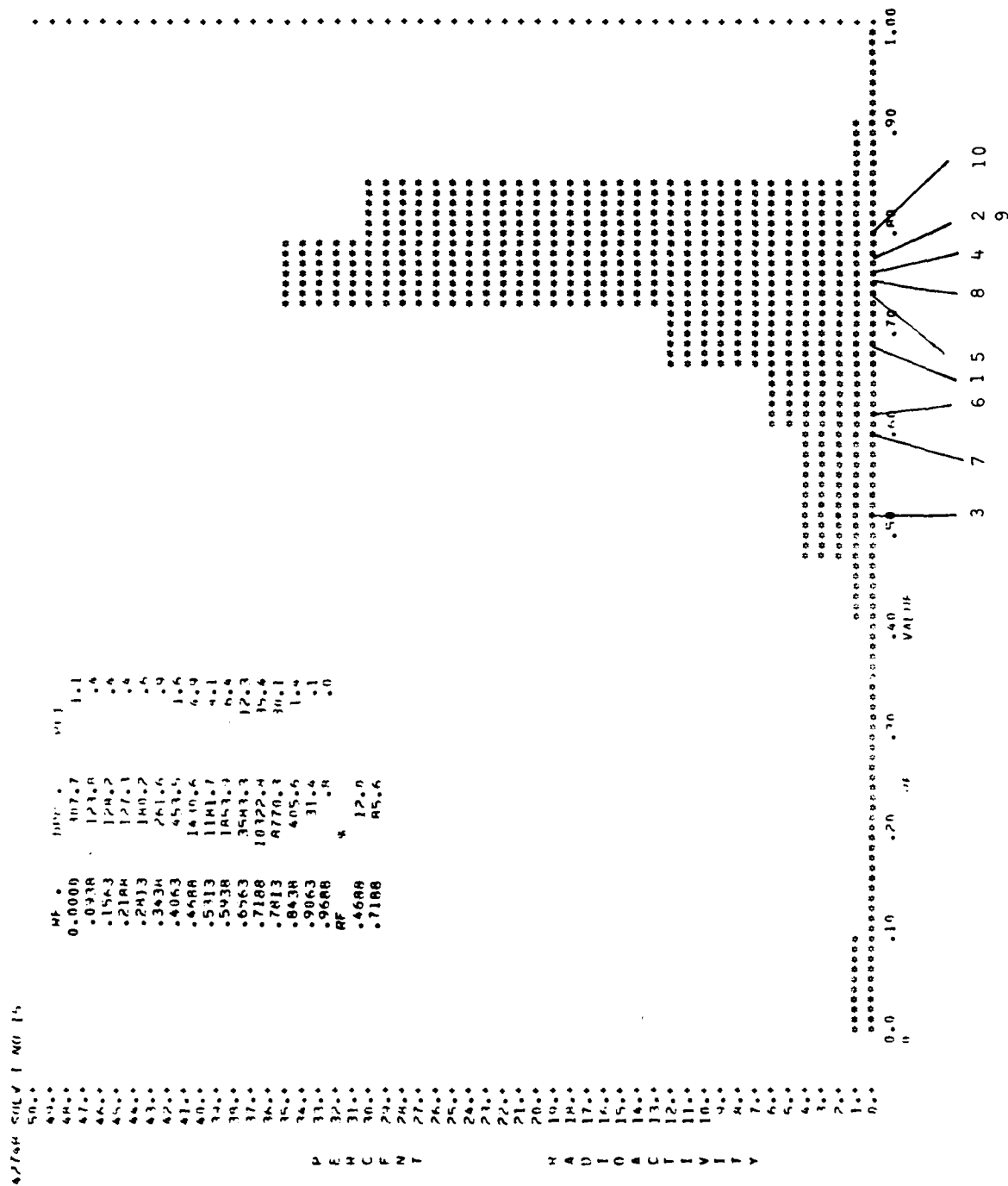


Figure 19-c-1: Intratracheal Instillation, Incubation with Water, Solvent I

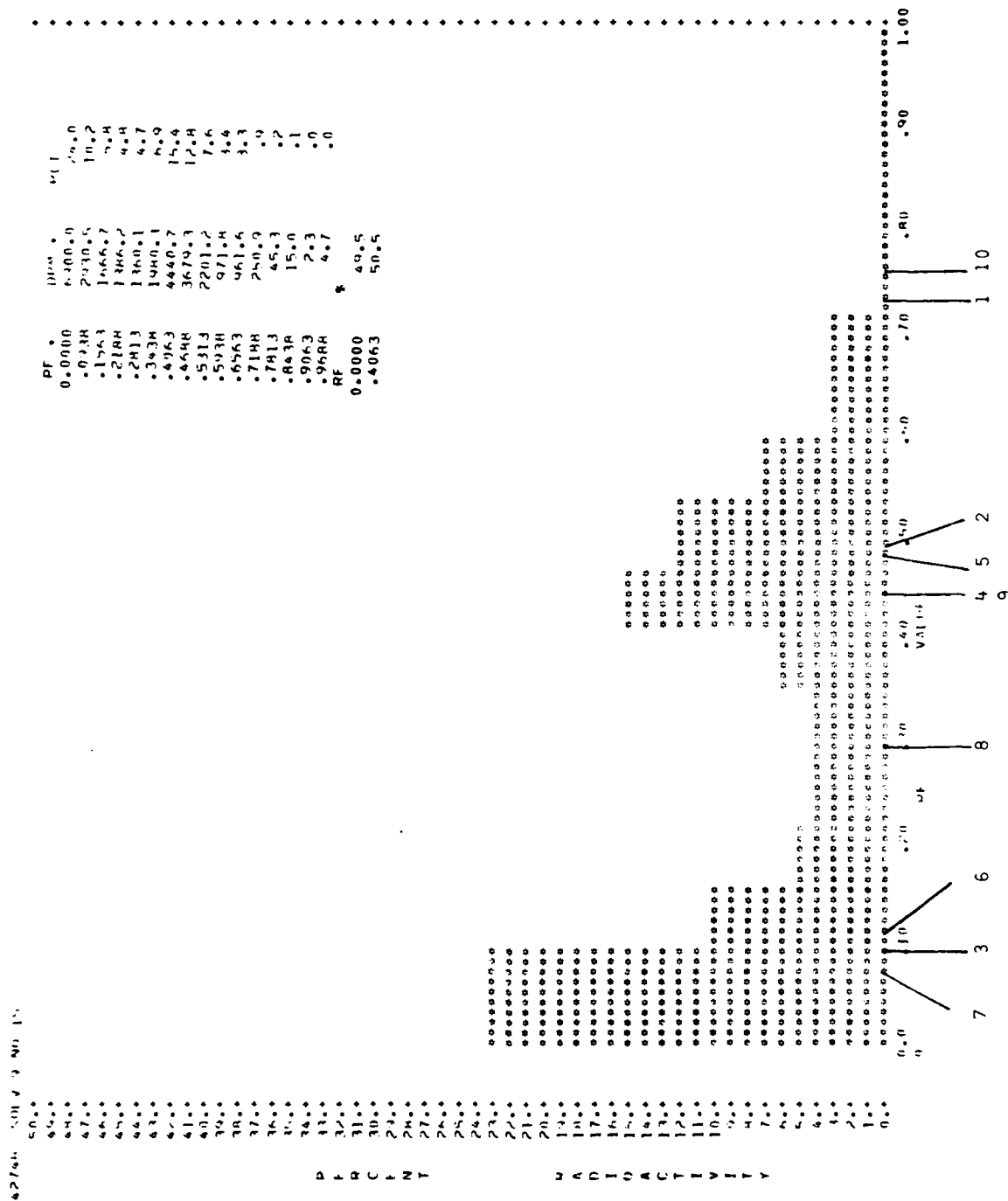


Figure 19-c-IX: Intratracheal Instillation, Incubation with Water, Solvent IX

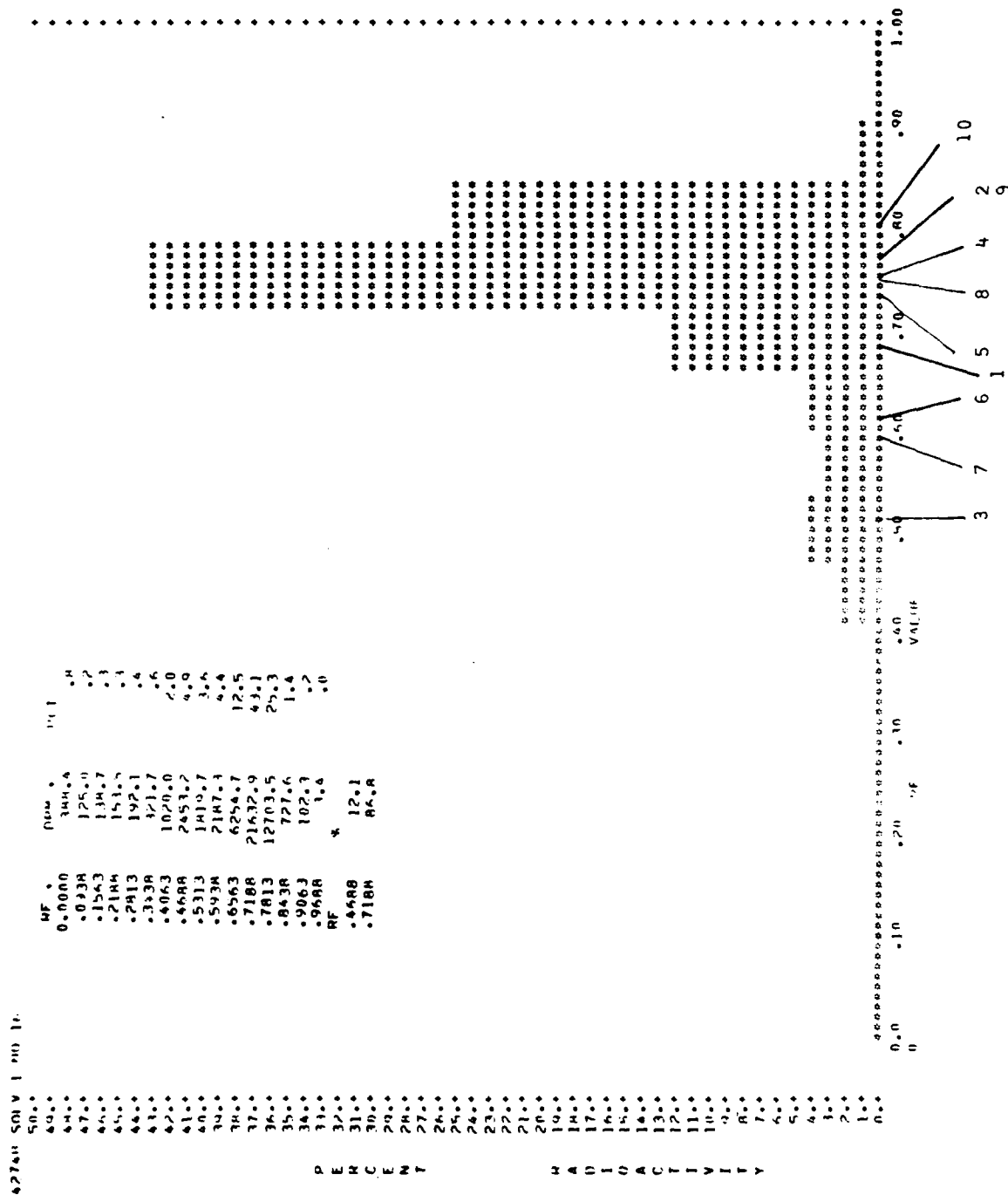


Figure 19-d-I: Intratracheal Instillation, Incubation with β -Glucuronidase, Solvent I

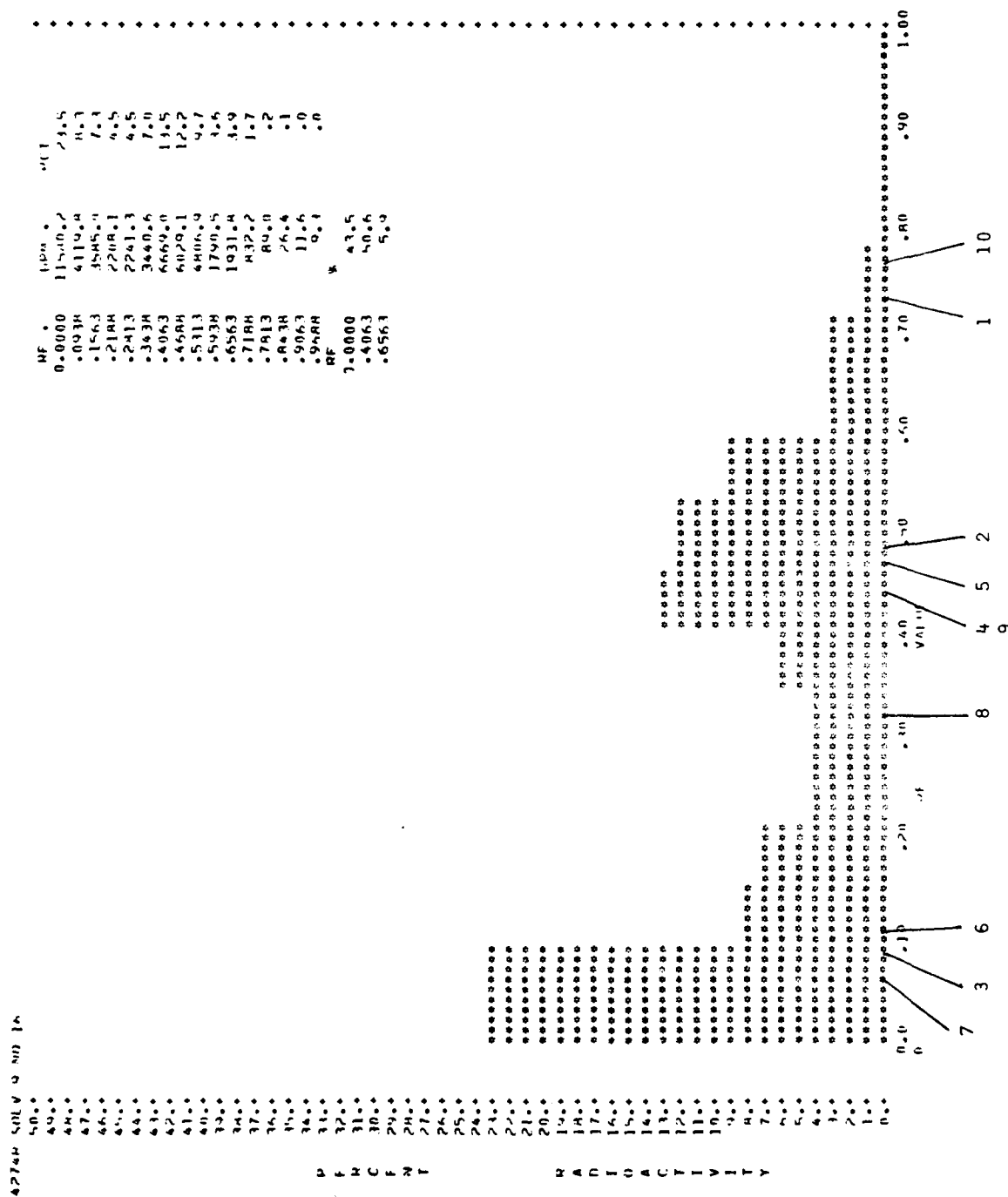


Figure 19-d-IX: Intratracheal Instillation, Incubation with β -Glucuronidase, Solvent IX

Figure 20: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Male Mice Treated Orally or Dermal with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 20 follows

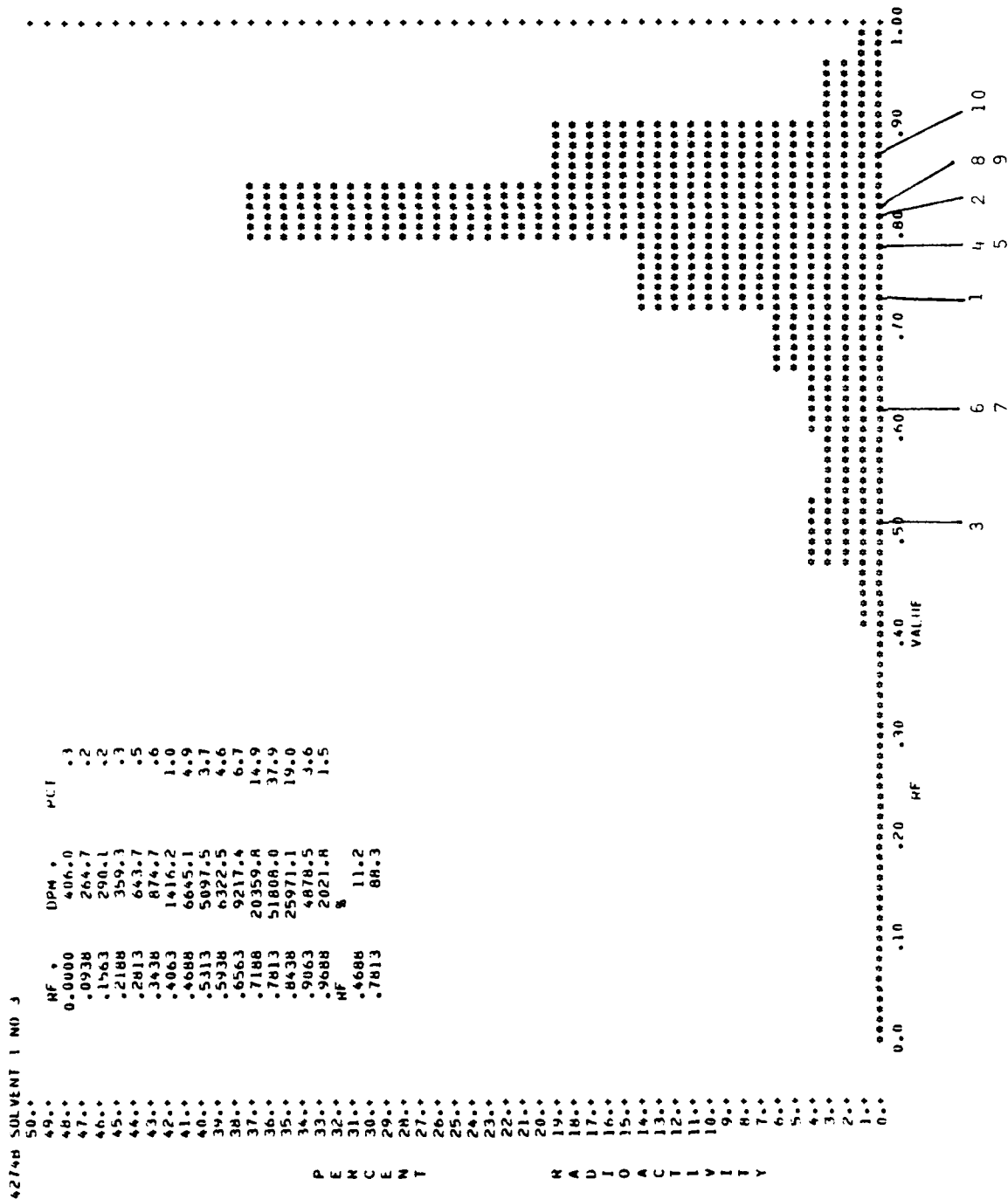


Figure 20-a-I: Oral Treatment, Incubation with Water, Solvent I

4274H SOLVENT 9 NO. 3

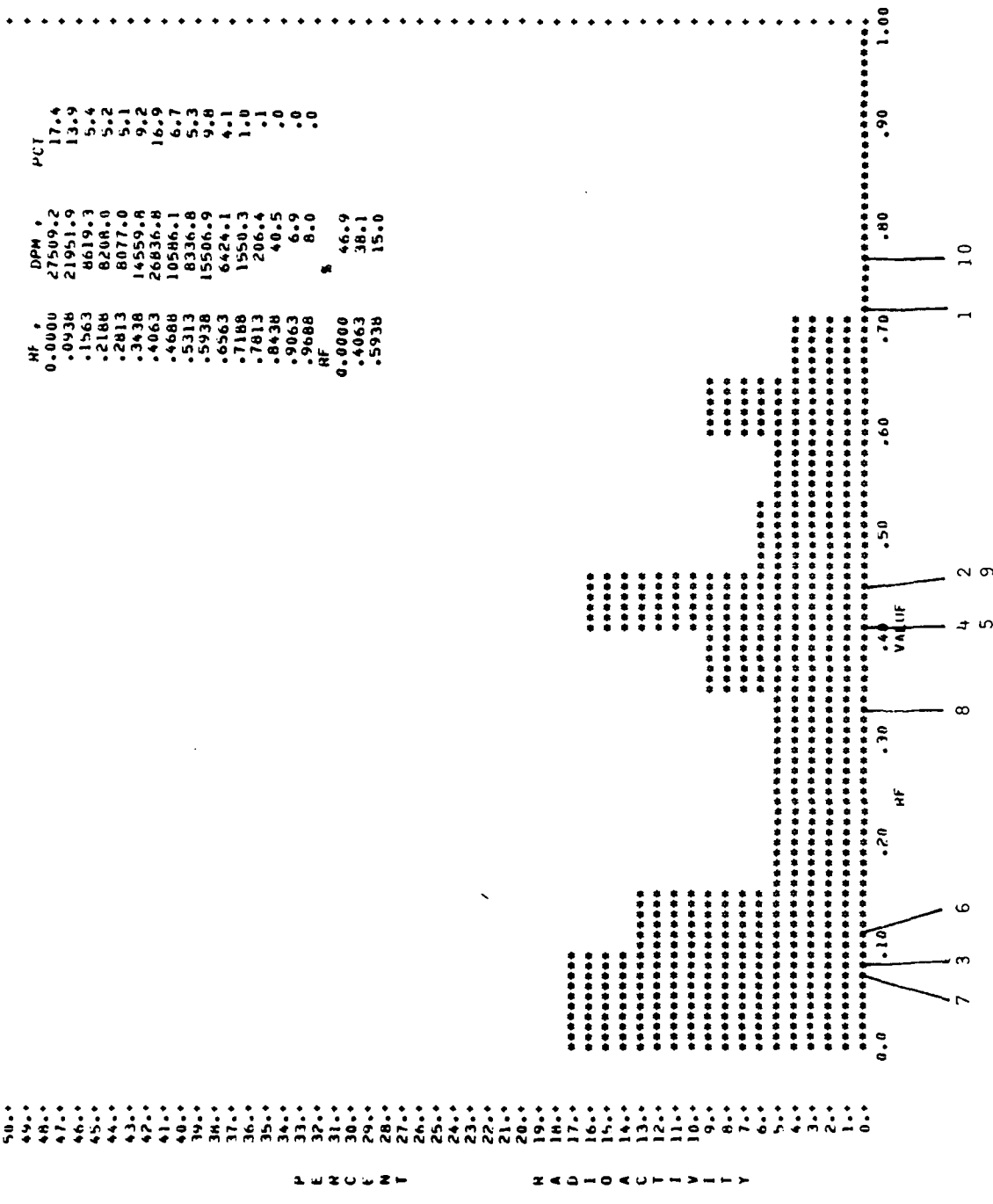


Figure 20-a-IX: Oral Treatment, Incubation with Water, Solvent IX

4274H SOLVENT 1 NO 4

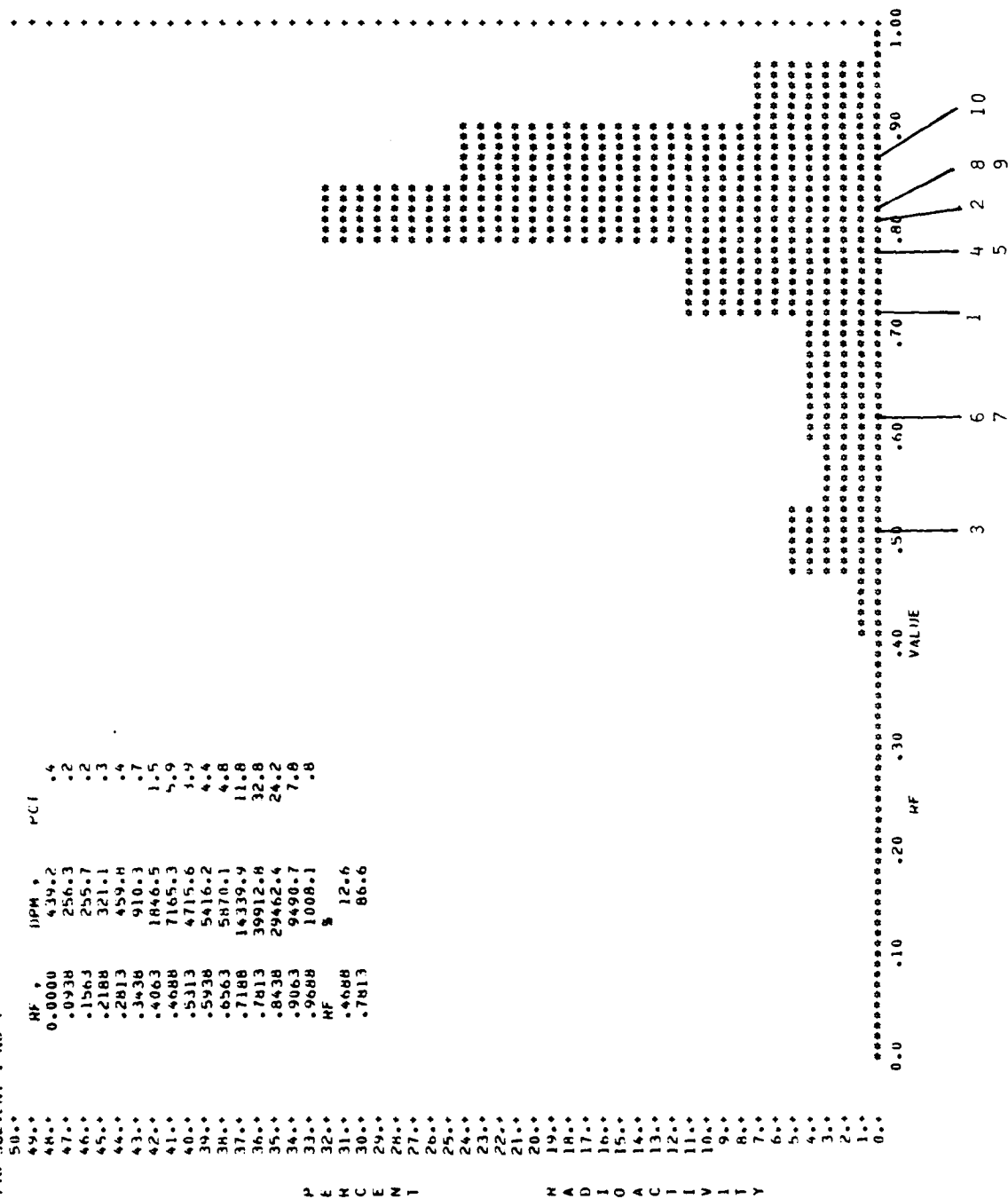


Figure 20-b-1: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

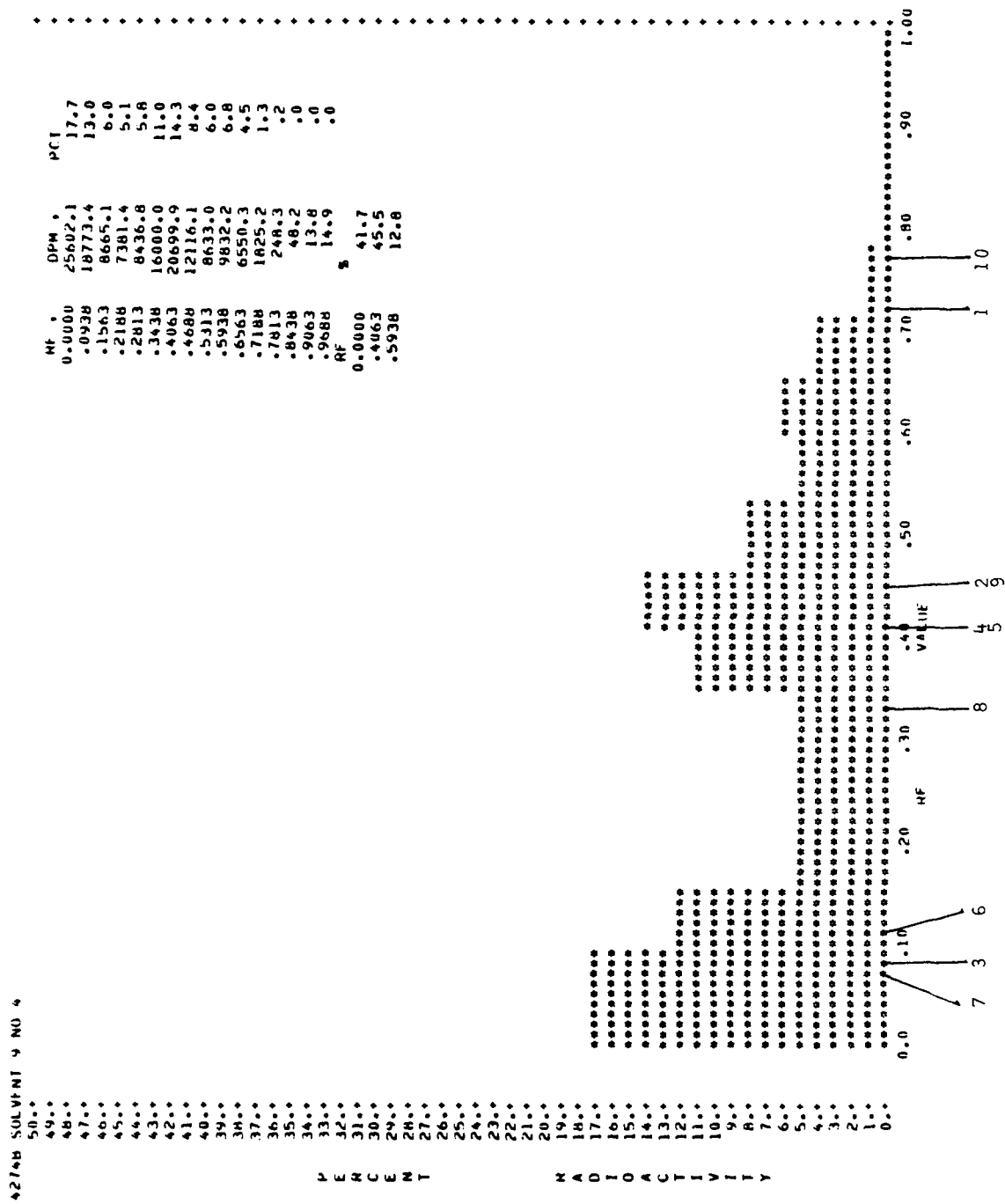


Figure 20-b-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

42746 SOLVENT 1 NO 1

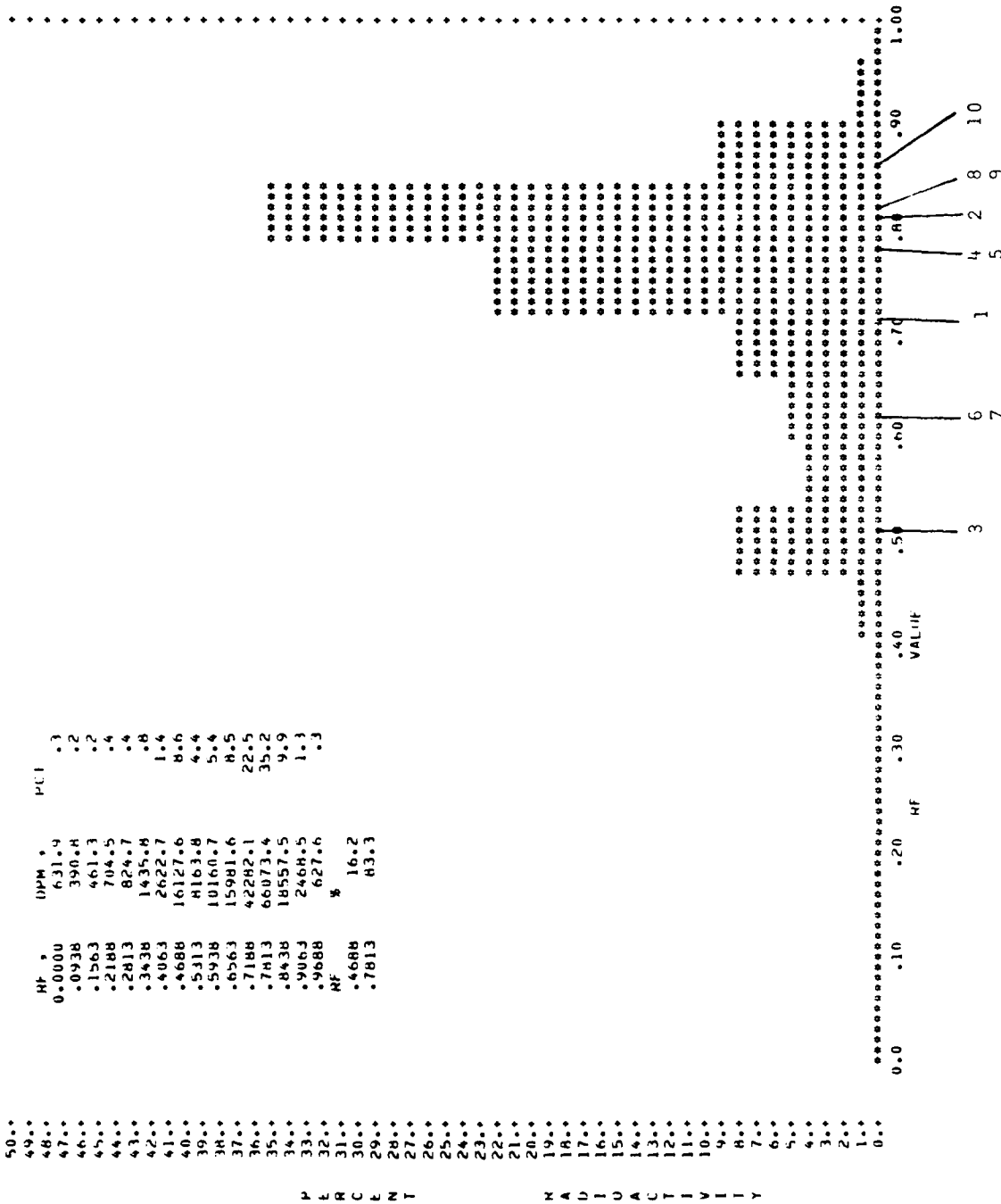


Figure 20-c-I: Dermal Application, Incubation with Water, Solvent I

4274H MOUSE HANBIT 006 MARCH 27 1978 SOLVENT 9 NO 1

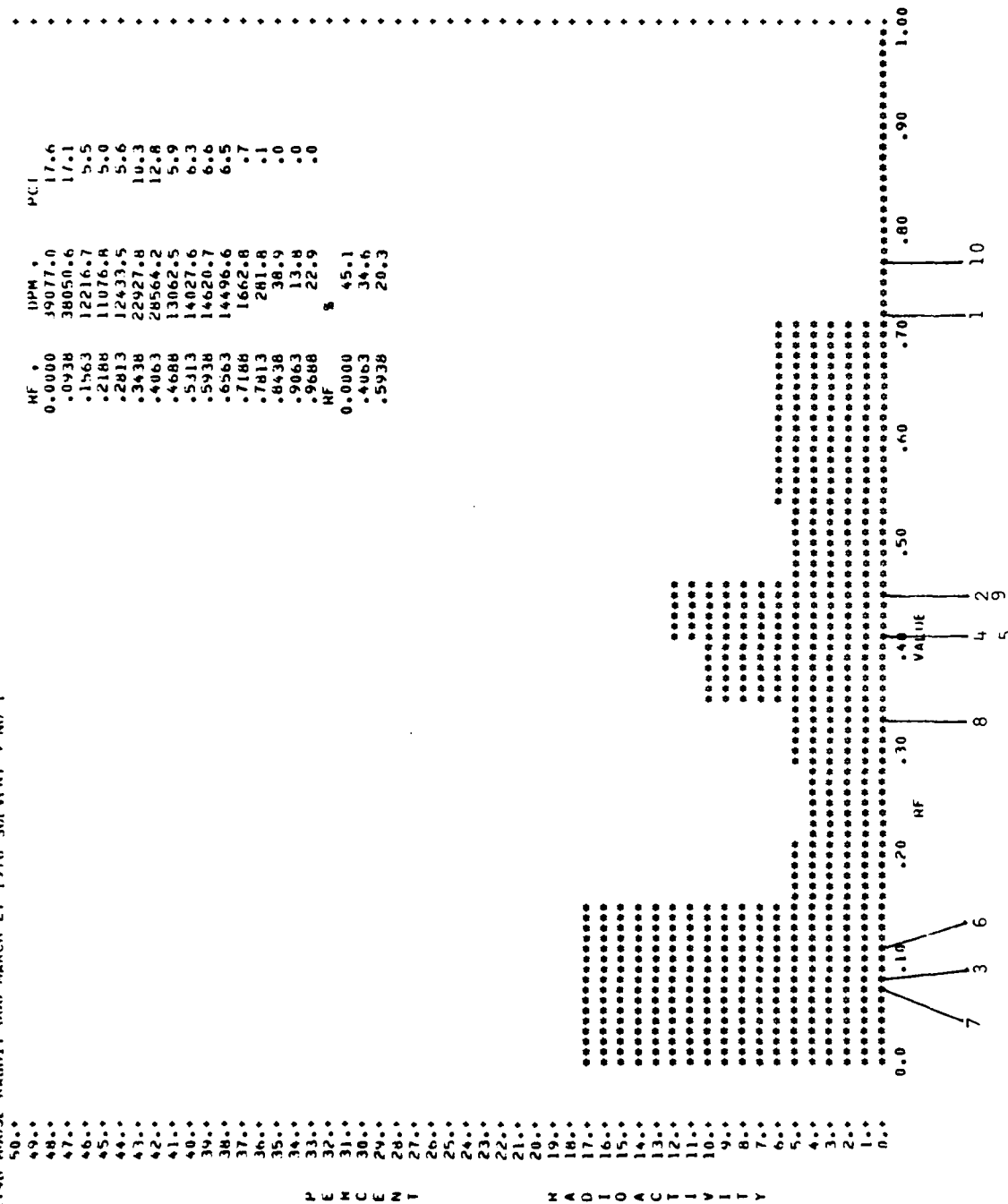


Figure 20-c-IX: Dermal Application, Incubation with Water, Solvent IX

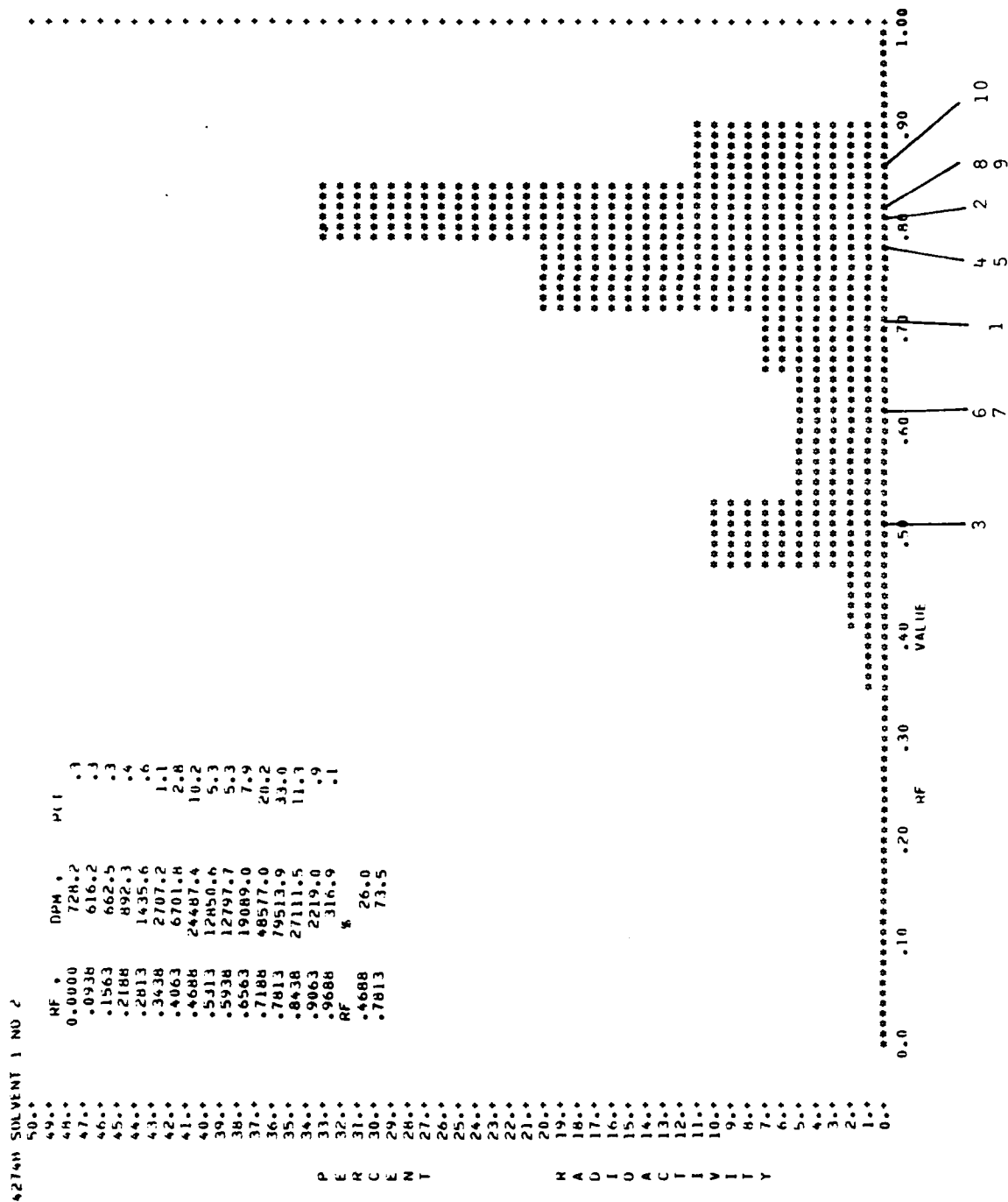


Figure 20-d-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

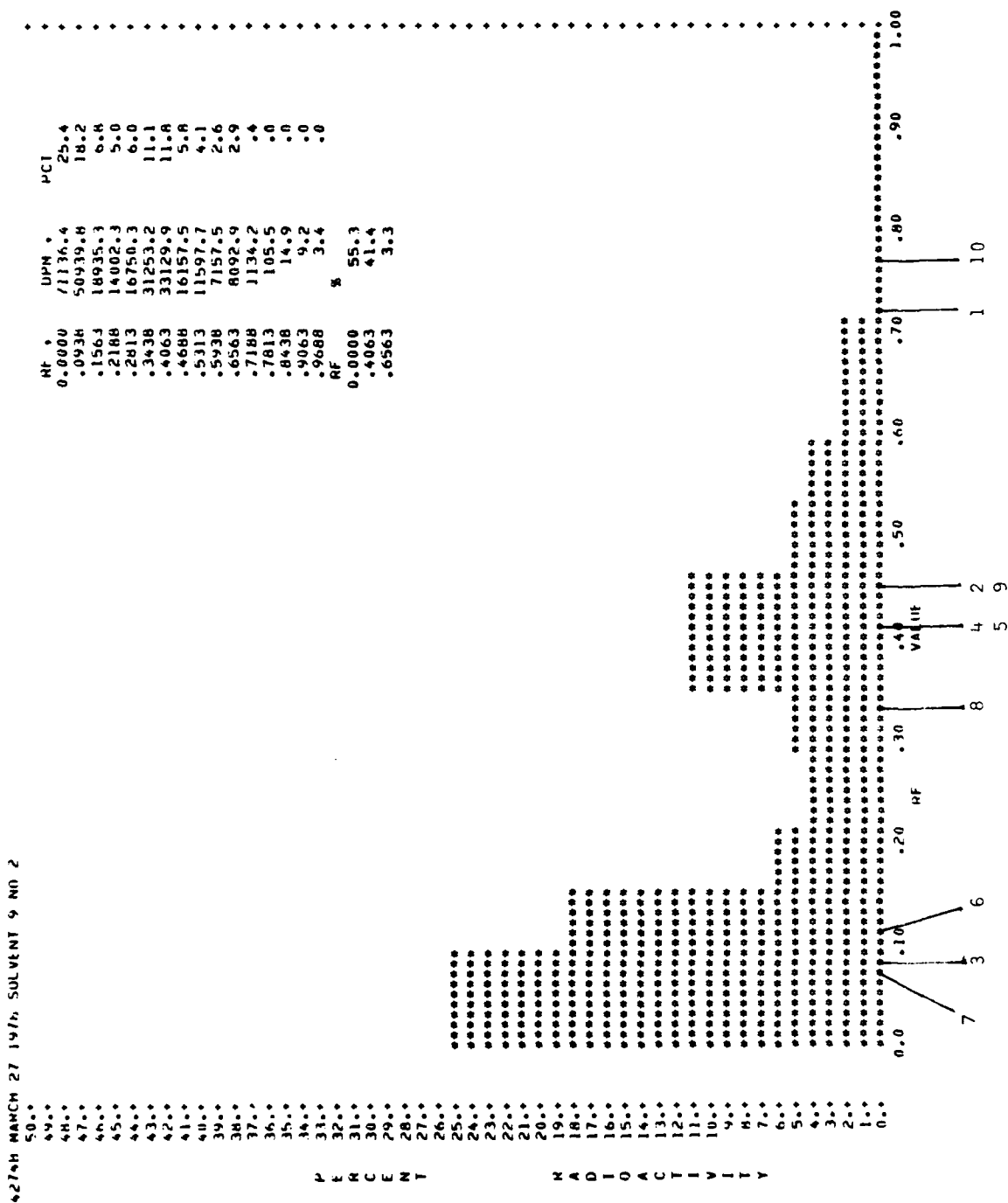


Figure 20-d-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

4274H SOL V 1 NO 1

P 50.0
 F 49.0
 R 48.0
 C 47.0
 E 46.0
 N 45.0
 T 44.0
 H 43.0
 A 42.0
 U 41.0
 I 40.0
 V 39.0
 I 38.0
 I 37.0
 I 36.0
 I 35.0
 I 34.0
 I 33.0
 I 32.0
 I 31.0
 I 30.0
 I 29.0
 I 28.0
 I 27.0
 I 26.0
 I 25.0
 I 24.0
 I 23.0
 I 22.0
 I 21.0
 I 20.0
 I 19.0
 I 18.0
 I 17.0
 I 16.0
 I 15.0
 I 14.0
 I 13.0
 I 12.0
 I 11.0
 I 10.0
 I 9.0
 I 8.0
 I 7.0
 I 6.0
 I 5.0
 I 4.0
 I 3.0
 I 2.0
 I 1.0
 I 0.0

RF .0000
 DPM .121.7
 PCT .4
 .0938
 .1563
 .2188
 .2813
 .3438
 .4063
 .4688
 .5313
 .5938
 .6563
 .7188
 .7813
 .8438
 .9063
 .9688
 RF .4688
 DPM .12.9
 PCT .5.1
 .0938
 .1563
 .2188
 .2813
 .3438
 .4063
 .4688
 .5313
 .5938
 .6563
 .7188
 .7813
 .8438
 .9063
 .9688
 RF .4688
 DPM .12.9
 PCT .5.1

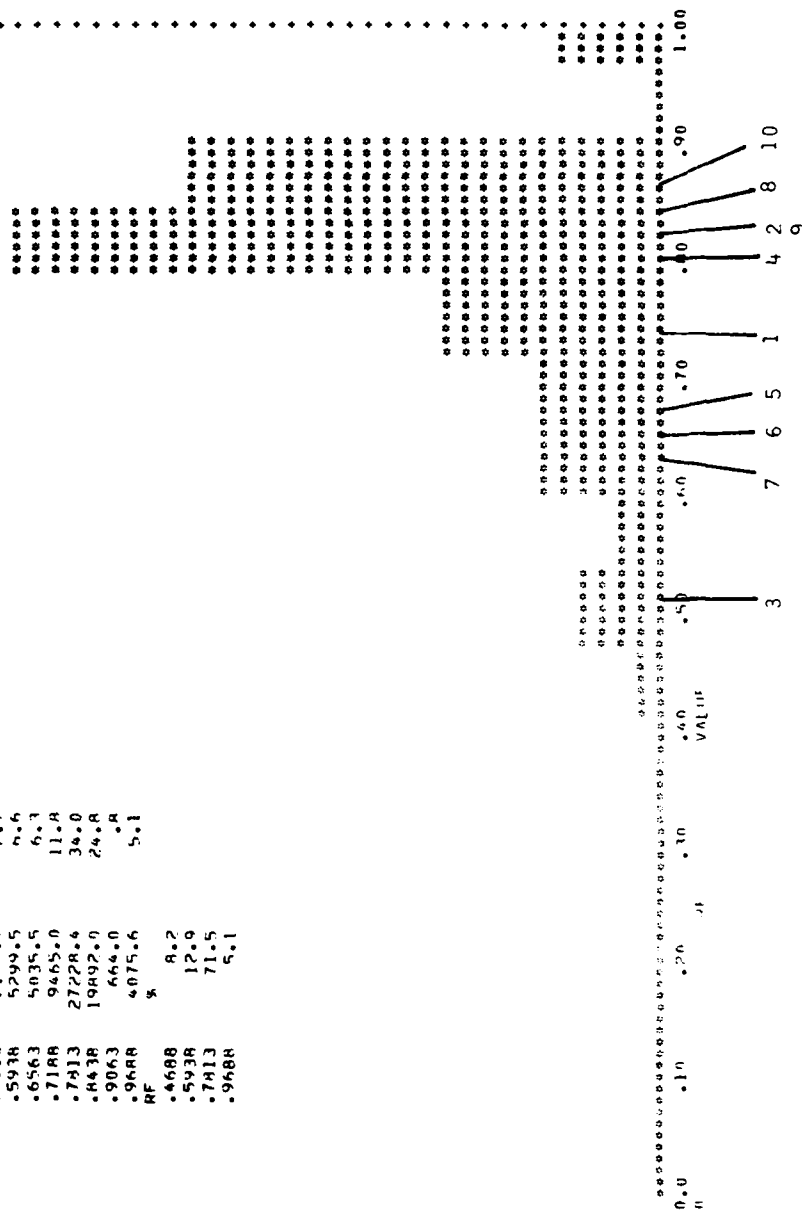


Figure 20-e-I: Oral Treatment, Incubation with Water, Solvent I

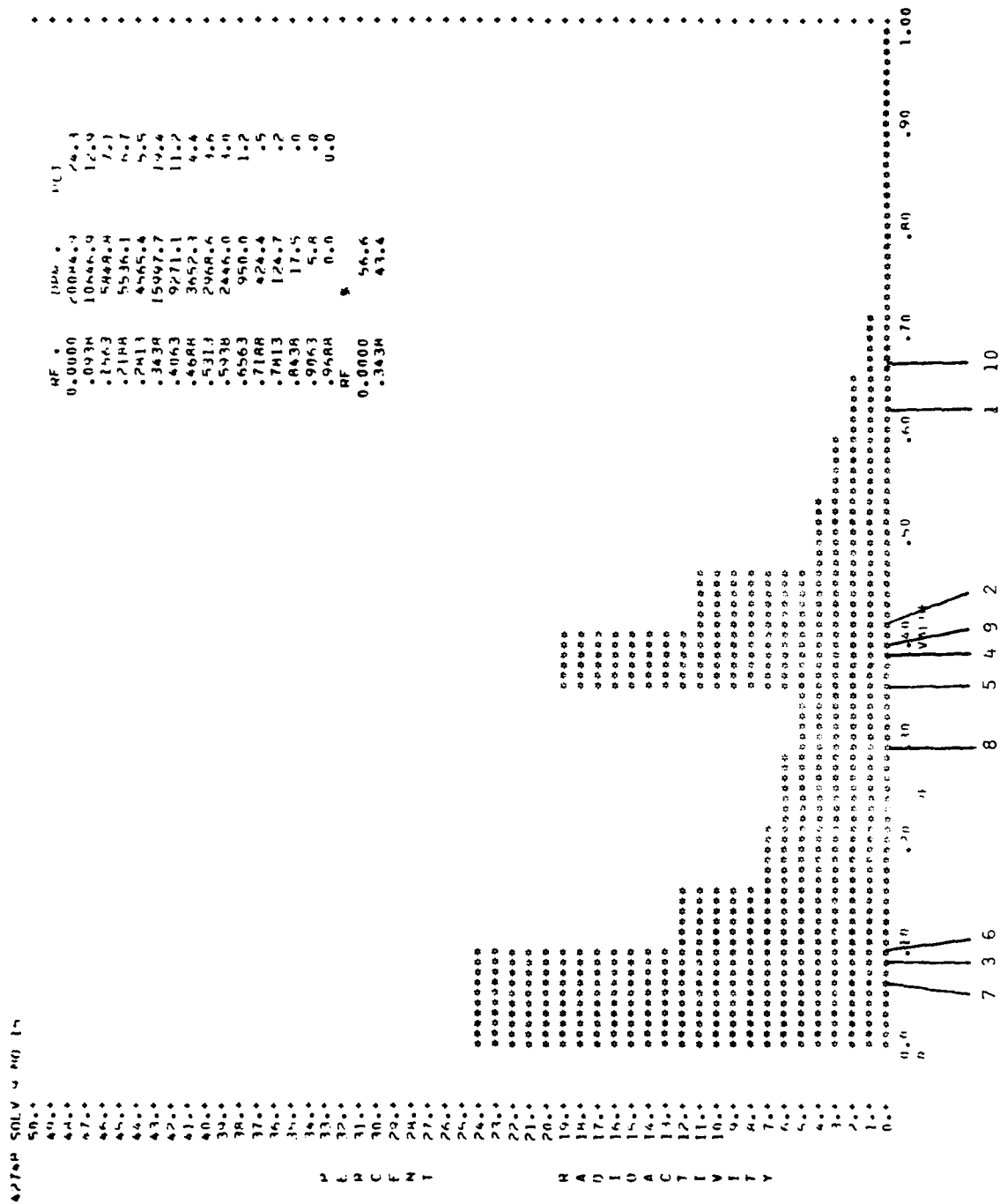


Figure 20-e-IX: Oral Treatment, Incubation with Water, Solvent IX

476M SOLV 1 NO 16

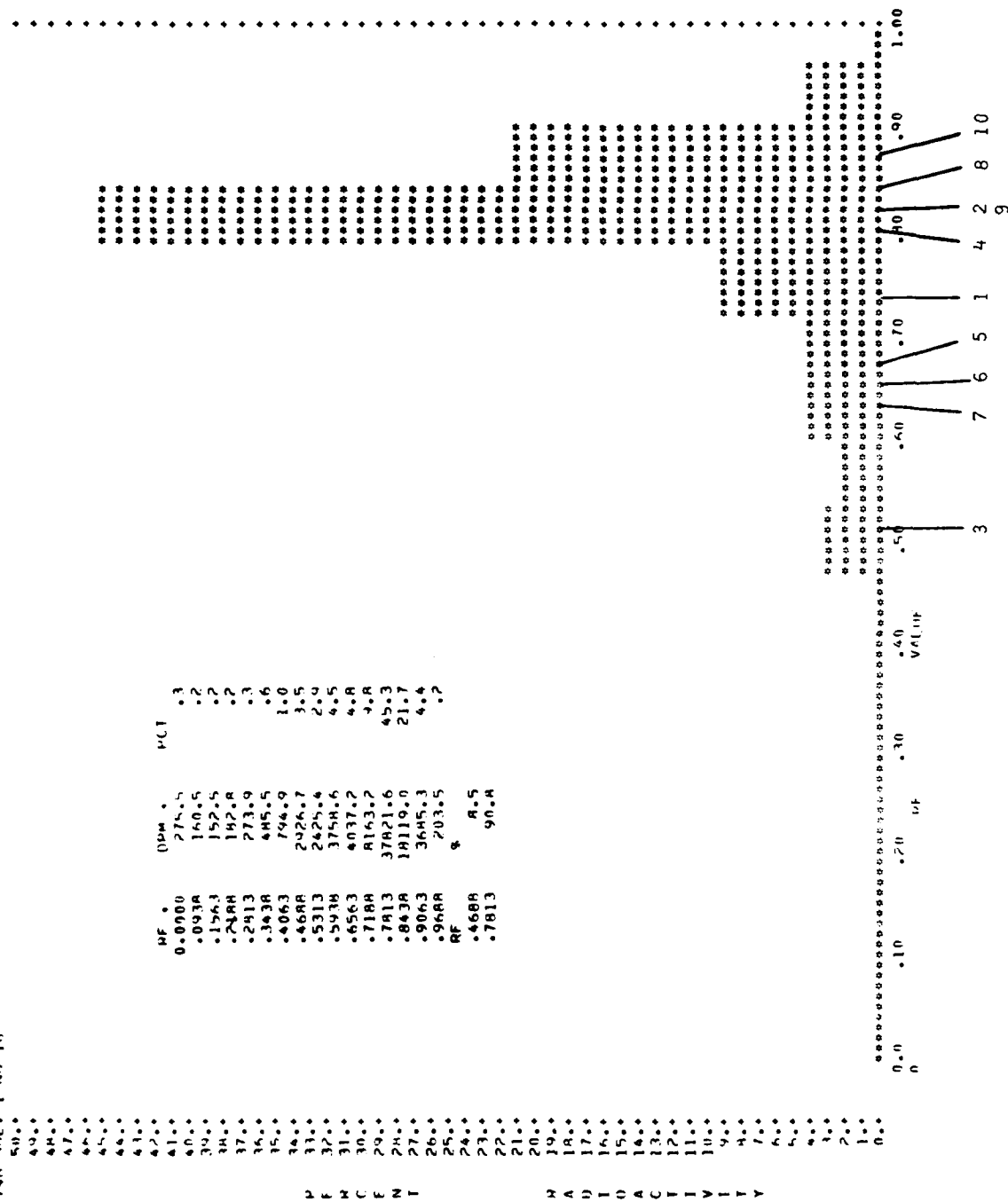


Figure 20-f-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

HF.	100%	%
0.0000	16044.4	14.2
0.0318	12510.6	14.2
1.563	10399.2	11.7
2.144	6627.6	5.2
2.813	5482.6	6.2
3.348	12636.9	16.4
4.063	15056.0	17.1
4.664	4493.0	5.7
5.513	2453.6	3.3
5.938	2296.9	2.6
6.563	776.7	0.9
7.188	361.3	.4
7.813	105.4	.1
8.438	24.4	.0
9.063	1.2	.0
9.688	3.5	.0
HF	%	
0.0000	69.3	
4.063	50.7	

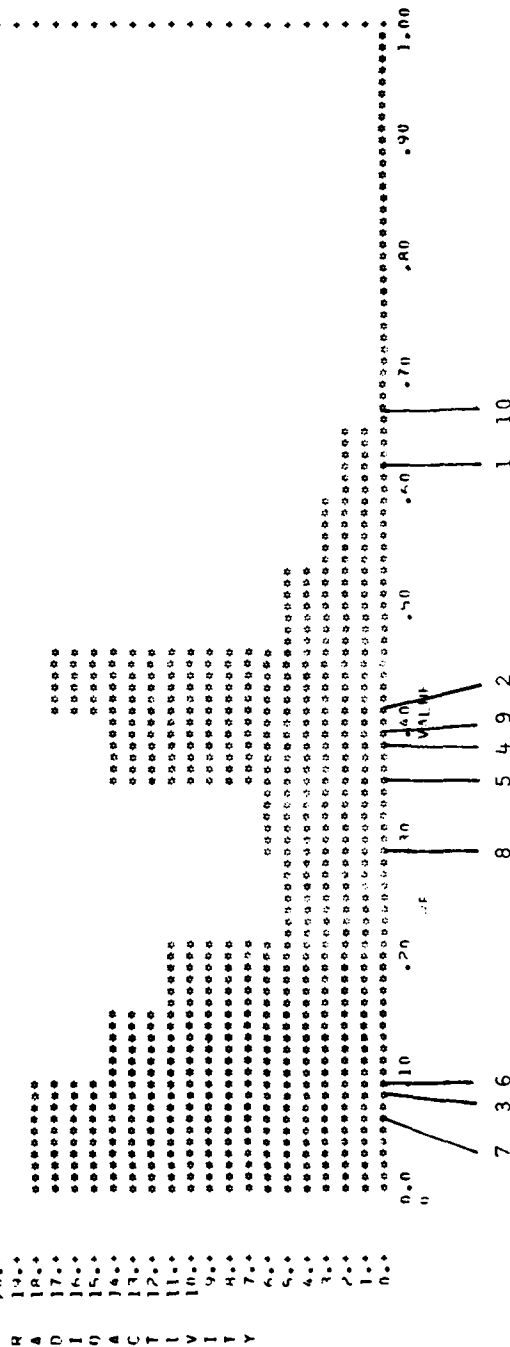


Figure 20-f-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

62748 SOLV 1 NO 13

50.0
40.0
44.0
47.0
46.0
45.0
44.0
43.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

U E R C E N T H A D U I O A C T I V I T Y

RF .
0.0000
.0934
.1563
.2184
.2413
.3434
.4063
.4684
.5113
.5938
.6563
.7184
.7813
.8434
.9063
.9684
RF
.4684
.7813

DPH .
146.3
63.7
45.1
46.4
215.6
142.9
453.4
2528.0
1452.3
2101.2
3245.3
6594.2
18426.3
6551.2
526.7
143.4
10.3
87.9

PCT .
.4
.1
.2
.2
.5
1.0
3.4
4.9
7.5
15.3
43.6
15.2
1.2
.3

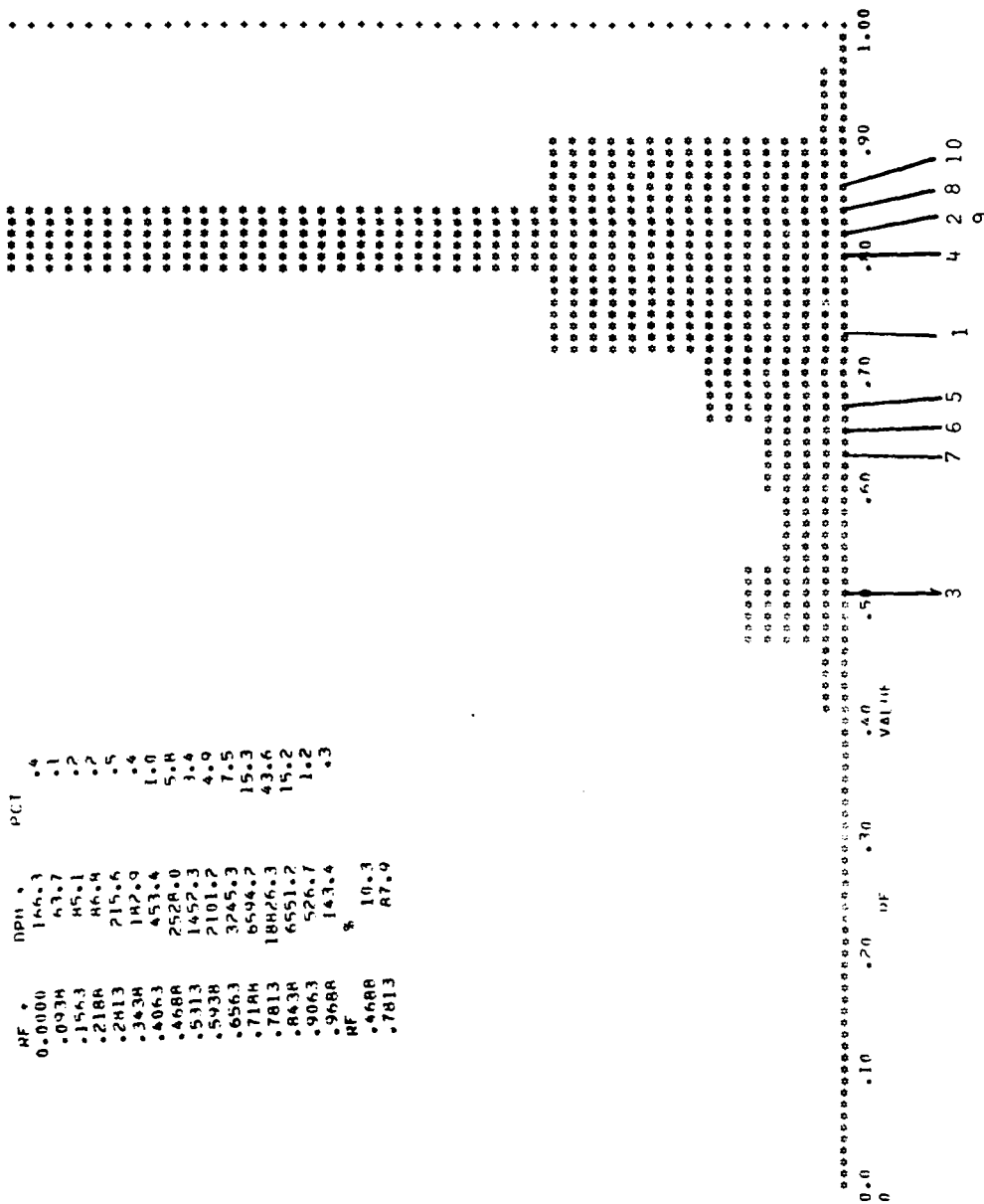
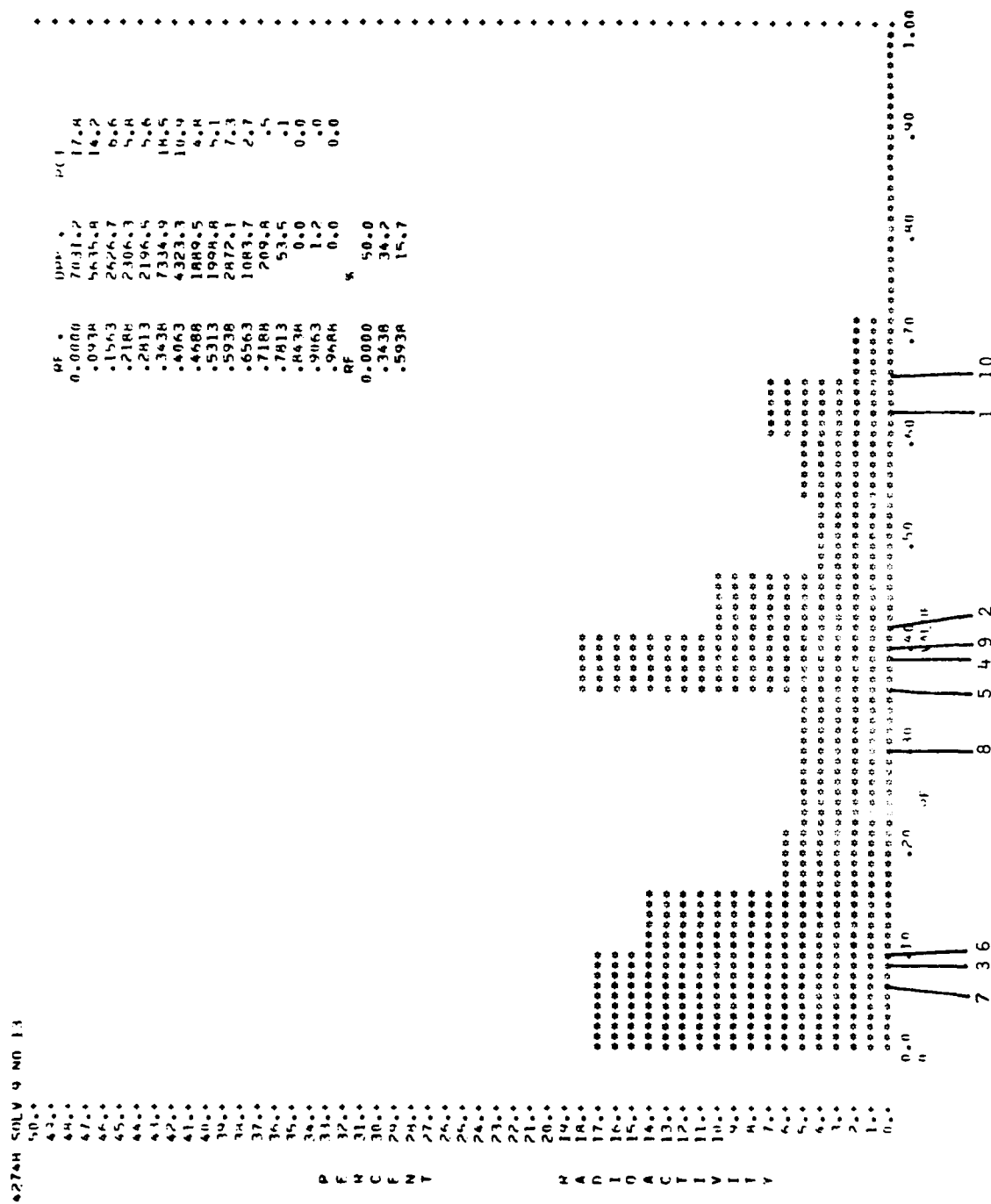


Figure 20-g-I: Dermal Application, Incubation with Water, Solvent I



4274H SOLV 1 NO 14

50.0

49.0

48.0

47.0

46.0

45.0

44.0

43.0

42.0

41.0

40.0

39.0

38.0

37.0

36.0

35.0

34.0

33.0

32.0

31.0

30.0

29.0

28.0

27.0

26.0

25.0

24.0

23.0

22.0

21.0

20.0

19.0

18.0

17.0

16.0

15.0

14.0

13.0

12.0

11.0

10.0

9.0

8.0

7.0

6.0

5.0

4.0

3.0

2.0

1.0

0.0

P F H R C F N T

R A D I O A C T I V E

4274H SOLV 1 NO 14

MF

0.0000

.0438

.1563

.2188

.2813

.3438

.4063

.4688

.5313

.5938

.6563

.7188

.7813

.8438

.9063

.9688

RF

.4688

.7813

UPM

125.0

89.5

94.9

101.4

196.5

274.3

719.6

2303.4

1659.7

1934.0

2350.3

4544.5

18044.3

10927.2

641.0

211.0

*

12.1

87.4

PCI

.3

.2

.2

.4

.6

1.6

5.2

3.8

4.4

5.3

10.3

40.8

24.7

1.5

.5

1.00

.90

.80

.70

.60

.50

.40

.30

.20

.10

0

VALIF

.40

.30

.20

.10

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Figure 20-h-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

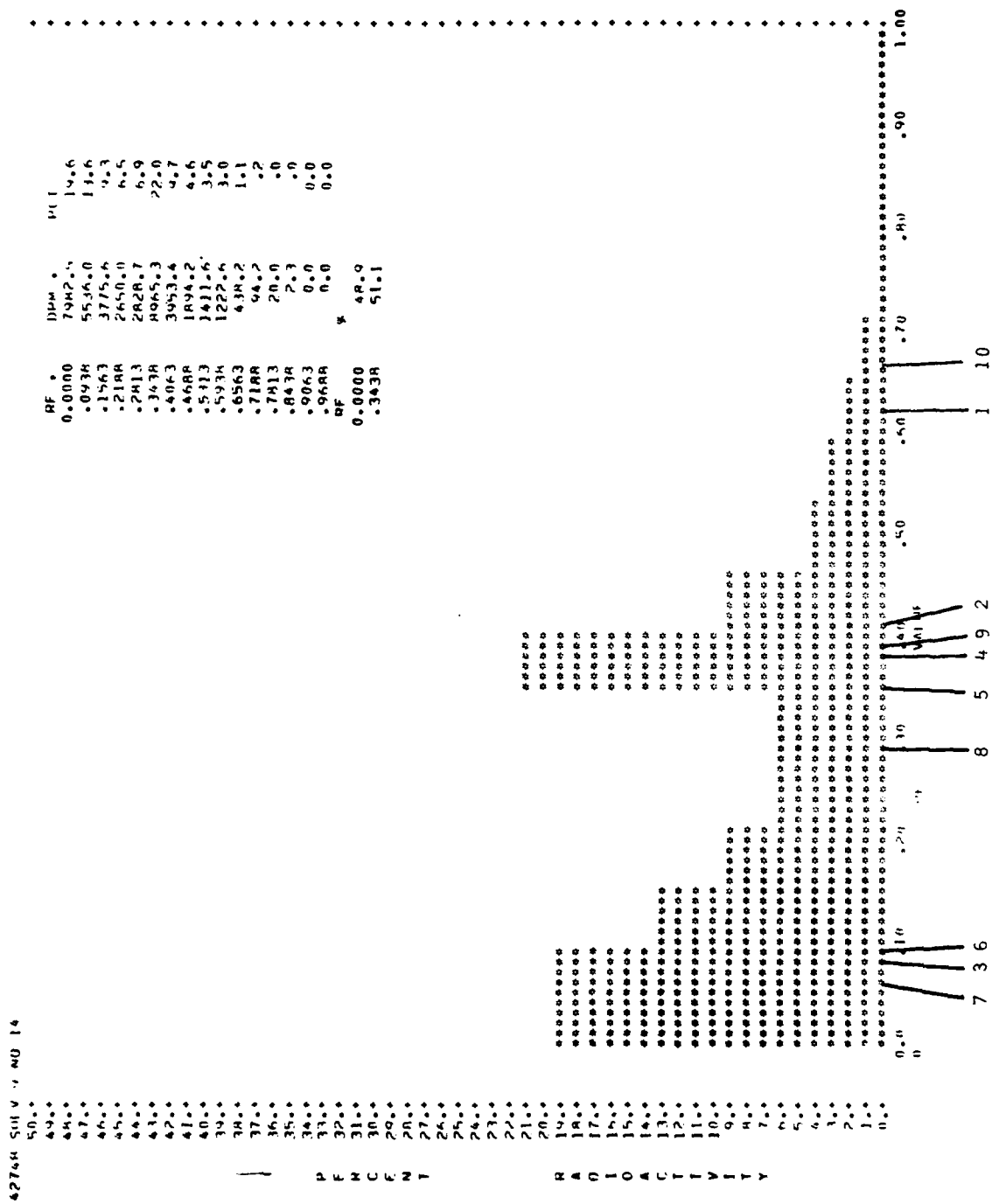


Figure 20-h-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

42748 SOLV 1 NO 4

50..	HF	DPM	PCT
49..	0.0000	65.1	.3
48..	.093M	63.6	.3
47..	.1563	62.4	.3
46..	.2188	60.6	.5
45..	.2813	175.6	.9
44..	.343M	236.0	1.2
43..	.4063	657.3	3.4
42..	.4688	1495.3	7.8
41..	.5313	1219.8	6.6
40..	.5938	1261.7	6.6
39..	.6563	3217.1	16.8
38..	.7188	7116.2	37.2
37..	.7813	2951.0	15.4
36..	.8438	487.2	2.5
35..	.9063	26.8	.1
34..	.9688	0.0	0.0
33..	RF	%	
32..			
31..	.4688	20.2	
30..	.7188	78.7	
29..			
28..			
27..			
26..			
25..			
24..			
23..			
22..			
21..			
20..			
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12..			
11..			
10..			
9..			
8..			
7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E R C N T

H A D I A C T I V

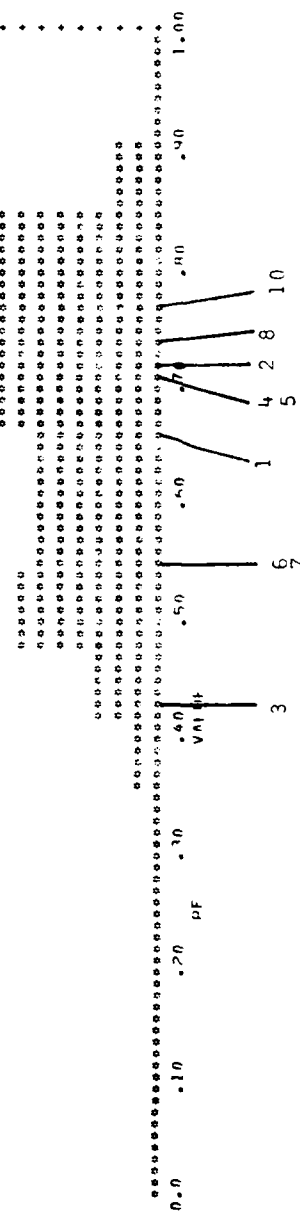


Figure 20-k-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

4274R SOLV 9 NO 4

P	50.00	RF	0.0000	DPW	4885.7	PCT	23.6
E	49.00		.0938		3201.6		15.4
H	48.00		.1563		1502.3		7.2
C	47.00		.2188		1264.0		6.1
E	46.00		.2413		1291.4		6.2
N	45.00		.3438		2087.2		10.1
T	44.00		.4063		3139.9		15.1
	43.00		.4688		1385.0		6.7
	42.00		.5313		672.1		3.2
	41.00		.5938		589.7		2.8
	40.00		.6563		313.5		1.5
	39.00		.7188		297.2		1.4
	38.00		.7813		84.8		.4
	37.00		.8438		16.3		.1
	36.00		.9063		0.0		0.0
	35.00		.9688		0.0		0.0
	34.00		RF		%		
	33.00		0.0000		52.3		
	32.00		.4063		47.6		

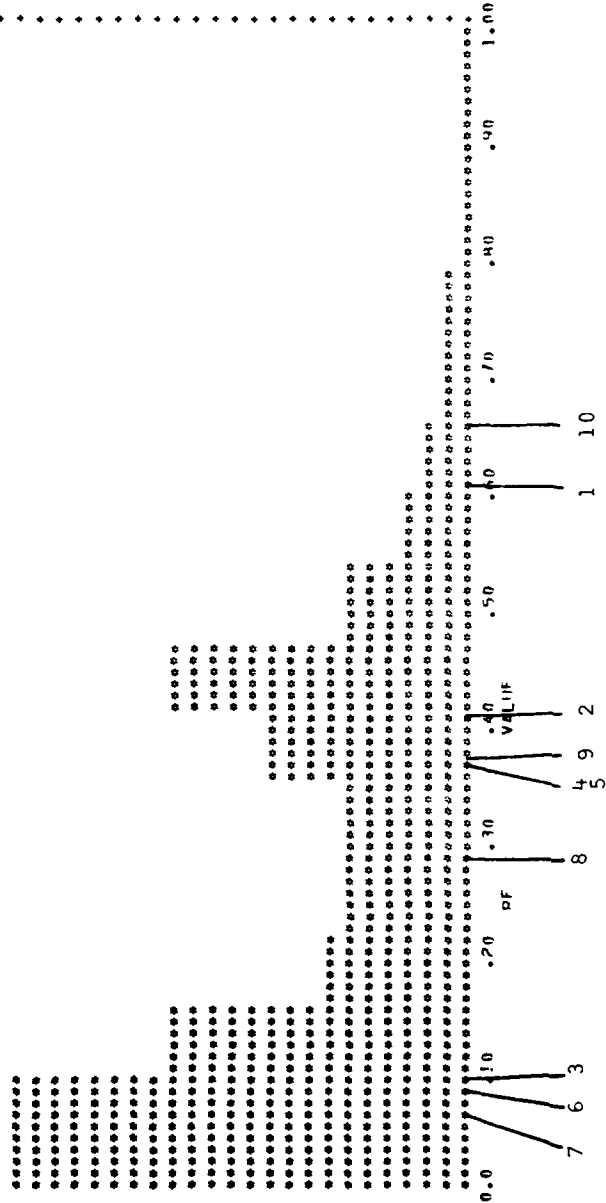


Figure 20-k-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

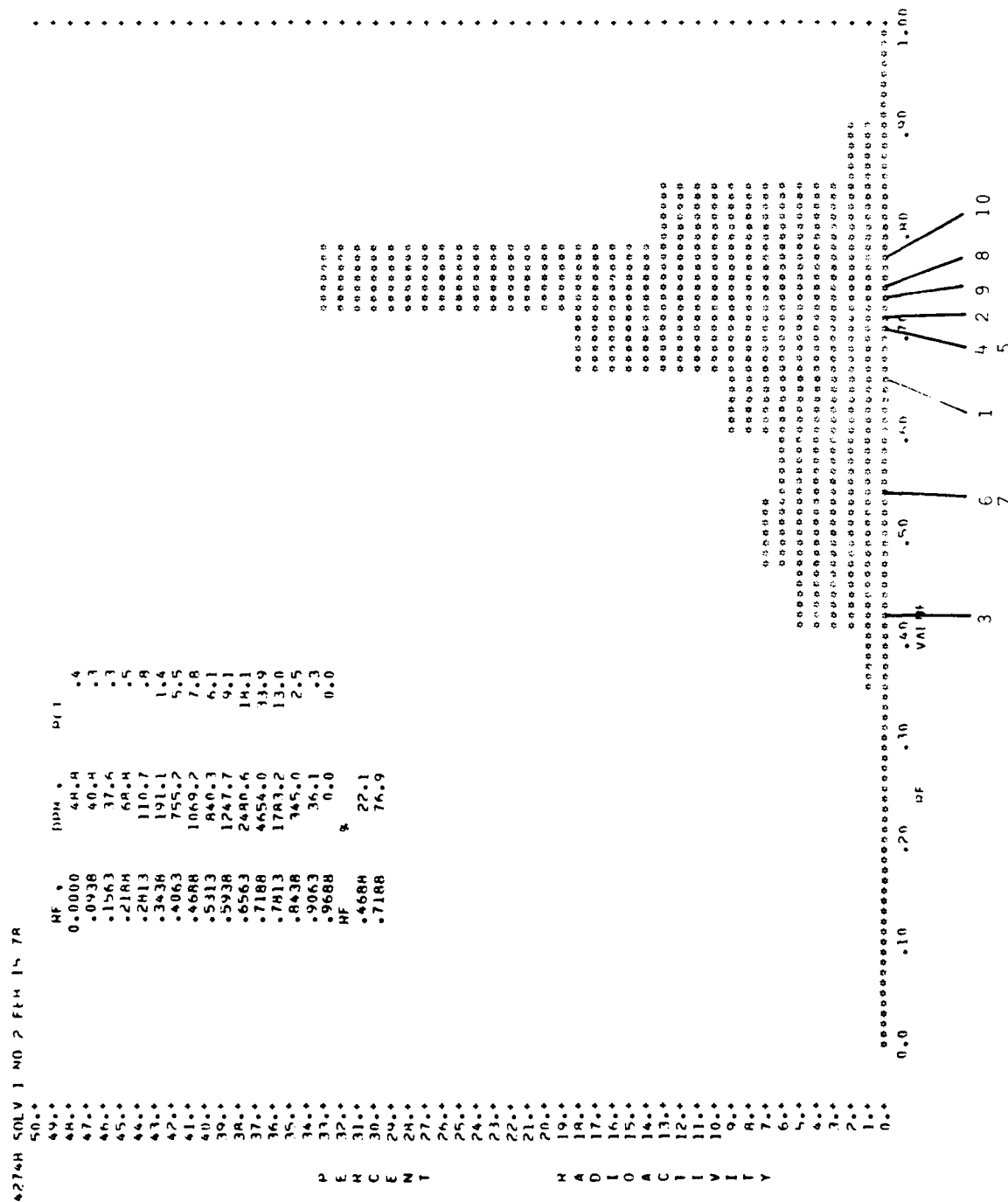


Figure 20-1-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

42748 SOLV 9 NO 2 FEB 15 78

50..	RF	DPM	PCI
49..	0.0000	2798.4	23.2
48..	.0938	2288.2	19.0
47..	.1563	1023.3	4.5
46..	.2188	767.4	6.4
45..	.2813	751.5	6.2
44..	.3438	1219.5	10.1
43..	.4063	1349.8	11.2
42..	.4688	682.6	5.7
41..	.5313	481.7	4.0
40..	.5938	287.2	2.4
39..	.6563	205.1	1.7
38..	.7188	144.5	1.2
37..	.7813	54.7	.5
36..	.8438	5.9	.0
35..	.9063	0.0	0.0
34..	.9688	0.0	0.0
33..	RF		
32..	0.0000	63.2	
31..	.4063	36.7	

P E H C E N T

R A D I O A C T I V I T Y

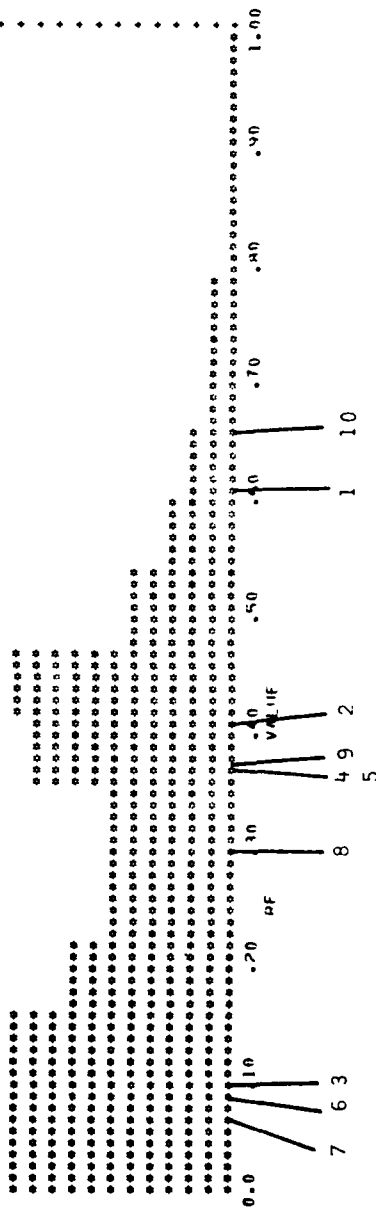


Figure 20-1-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

Figure 21: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Male Rabbits Treated Orally or Dermally with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 21 follows

4274H SOLV 1 #0 11

50.0
49.0
48.0
47.0
46.0
45.0
44.0
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

RF %
0.0000
0.038
0.163
0.218
0.281
0.343
0.406
0.468
0.531
0.593
0.656
0.718
0.781
0.843
0.906
0.968
RF %
14.7
31.5
41.0
52.1
64.7
100.0
247.2
340.3
532.7
1162.9
1204.0
5444.1
3308.1
364.3
65.4
2.3
14.5
84.9

PCT

0.3
0.2
0.1
0.4
0.5
0.7
2.1
6.2
4.4
4.5
4.8
40.0
24.3
7.7
0.5
0.0

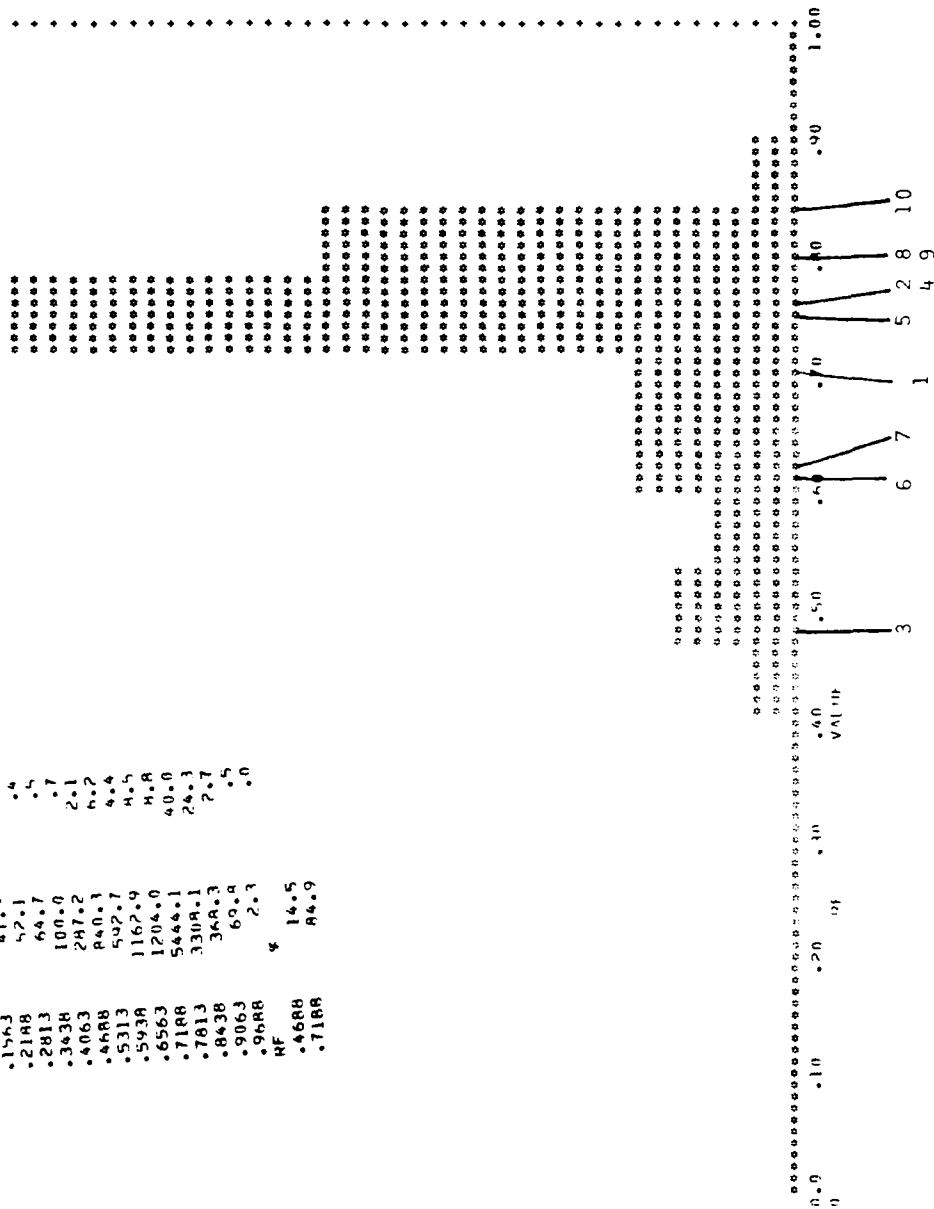


Figure 21-a-I: Oral Treatment, Incubation with Water, Solvent I

42766 SURV - NO 11

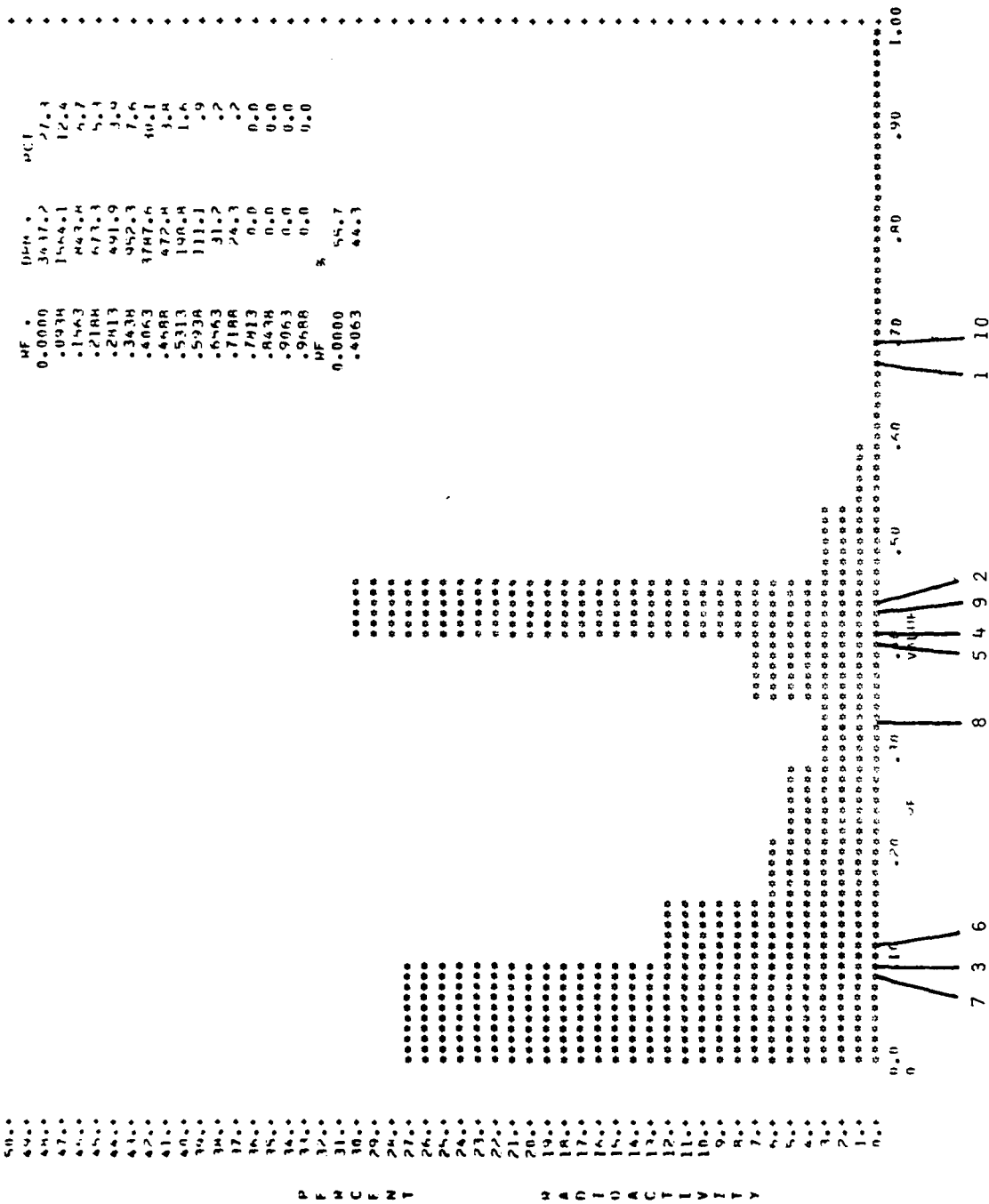


Figure 21-a-IX: Oral Treatment, Incubation with Water, Solvent IX

4274H ONLY 1 NO 12

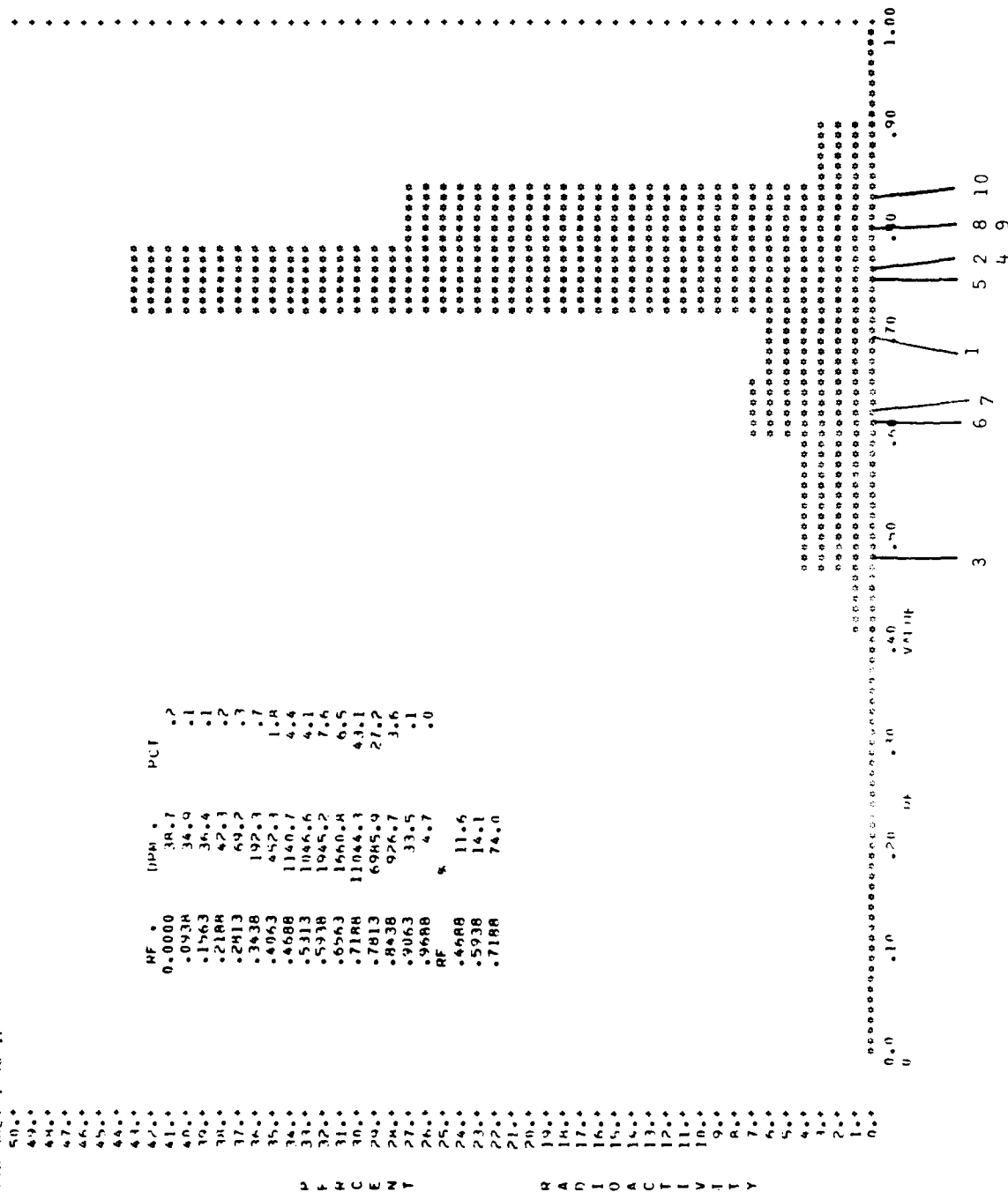


Figure 21-b-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

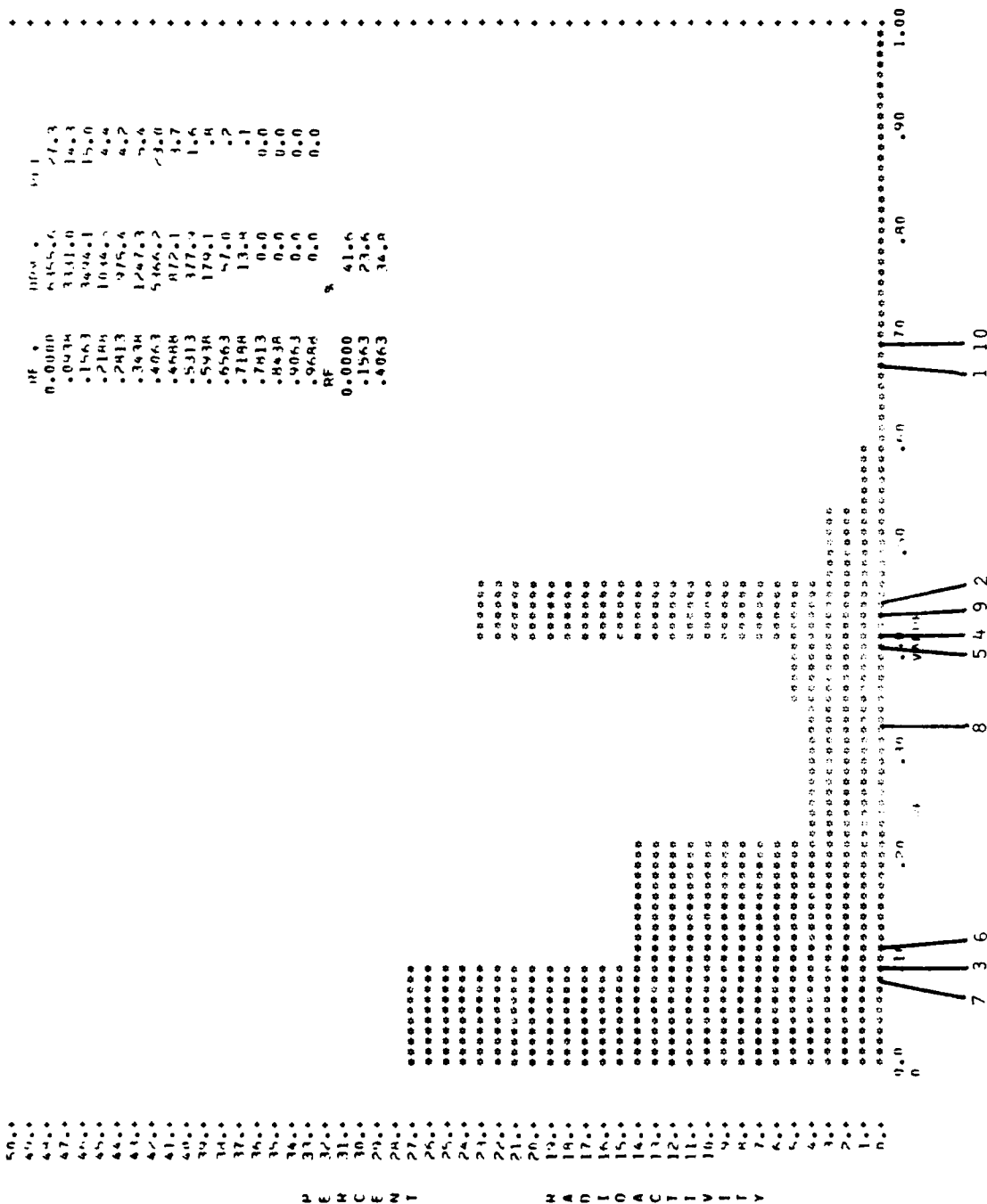


Figure 21-b-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

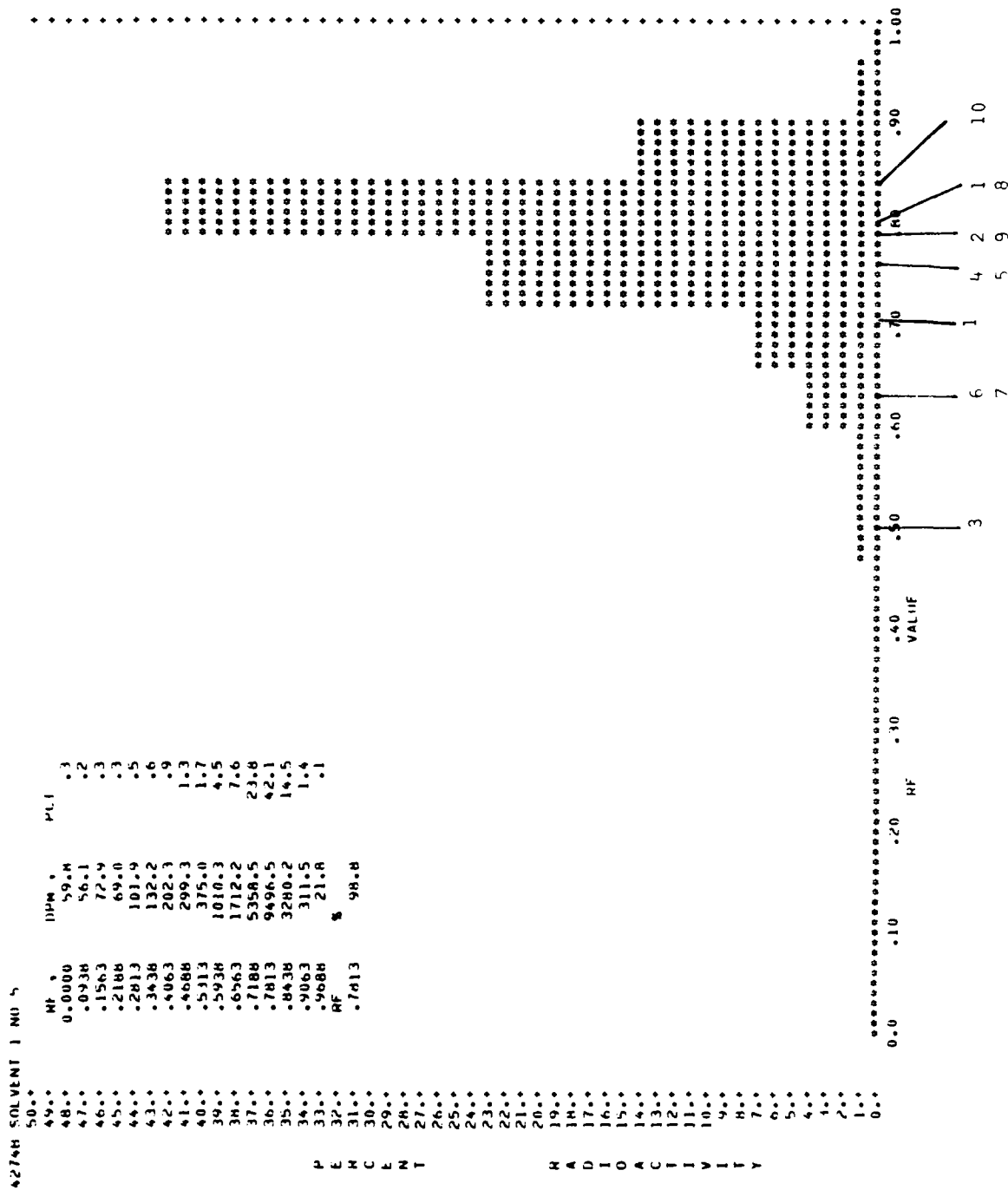


Figure 21-c-I: Dermal Application, Incubation with Water, Solvent I

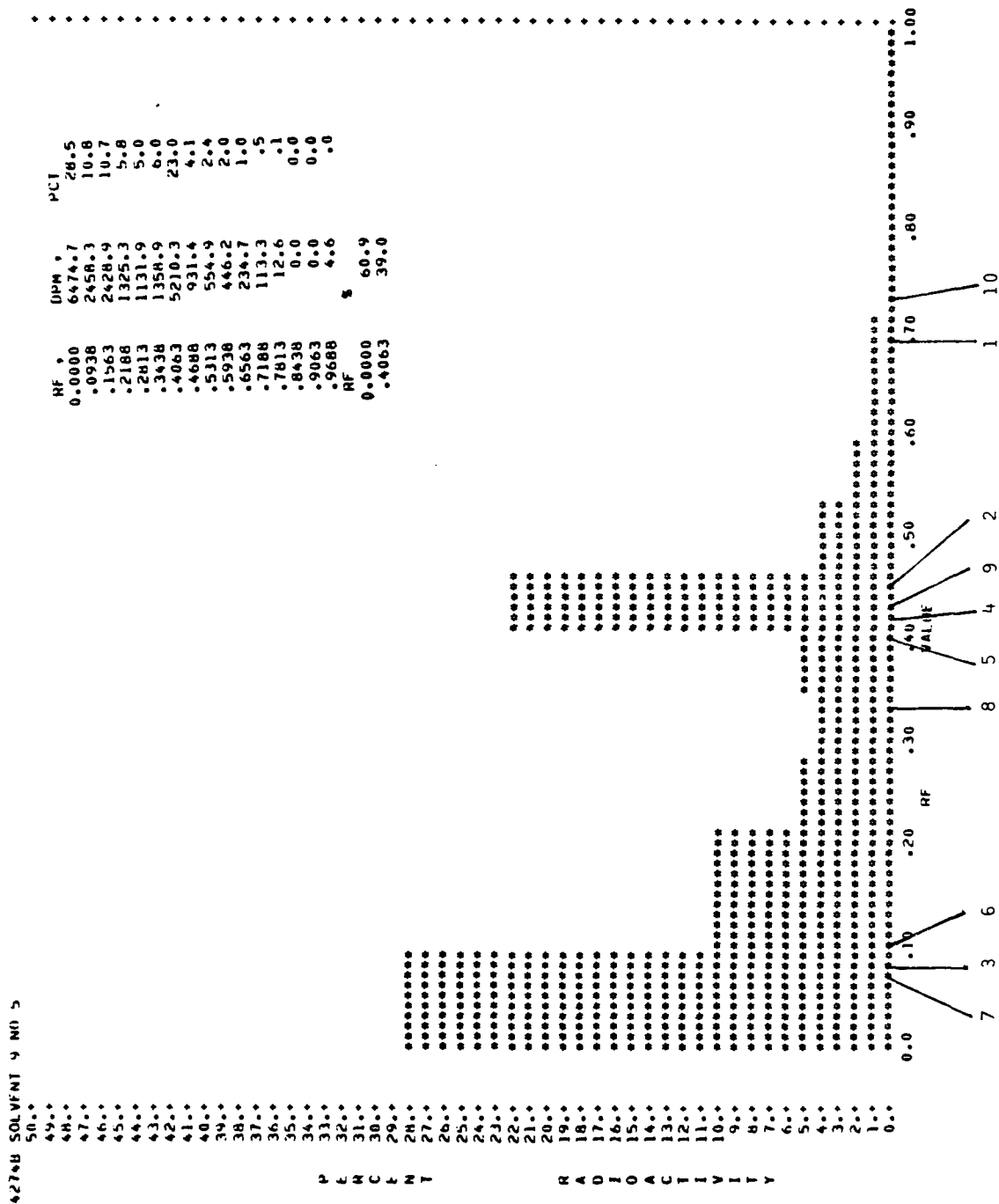


Figure 21-c-IX: Dermal Application, Incubation with Water, Solvent IX

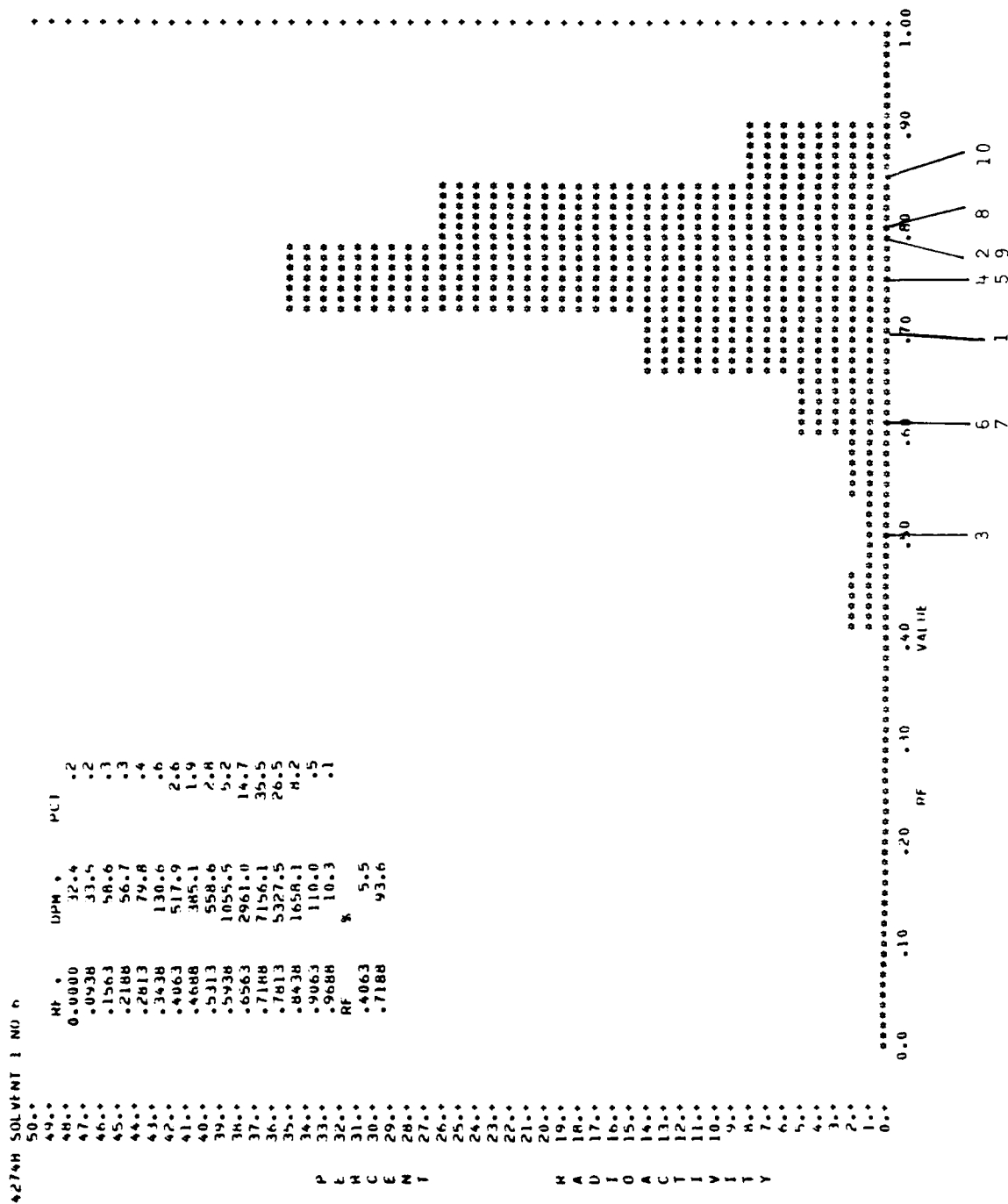


Figure 21-d-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

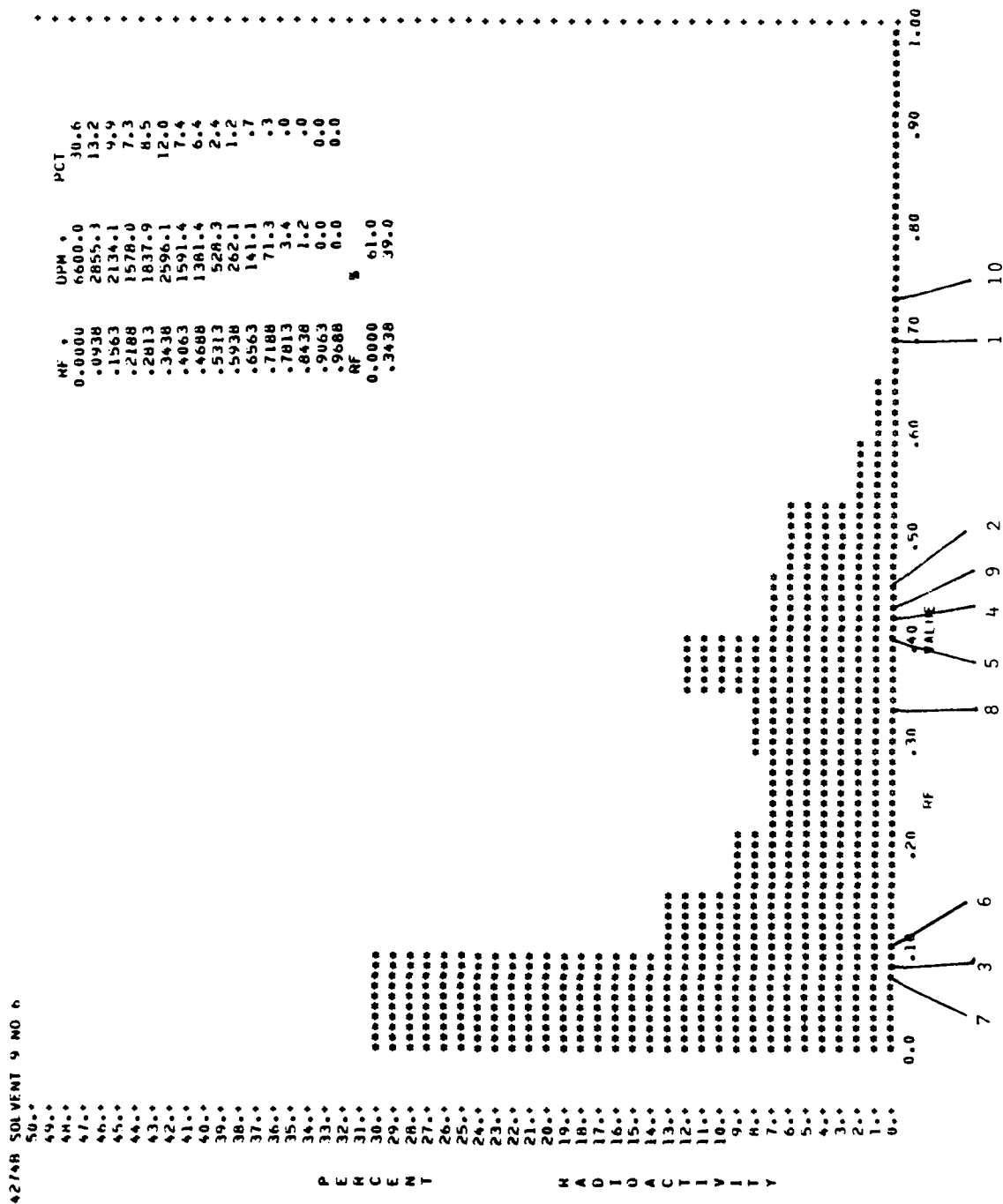
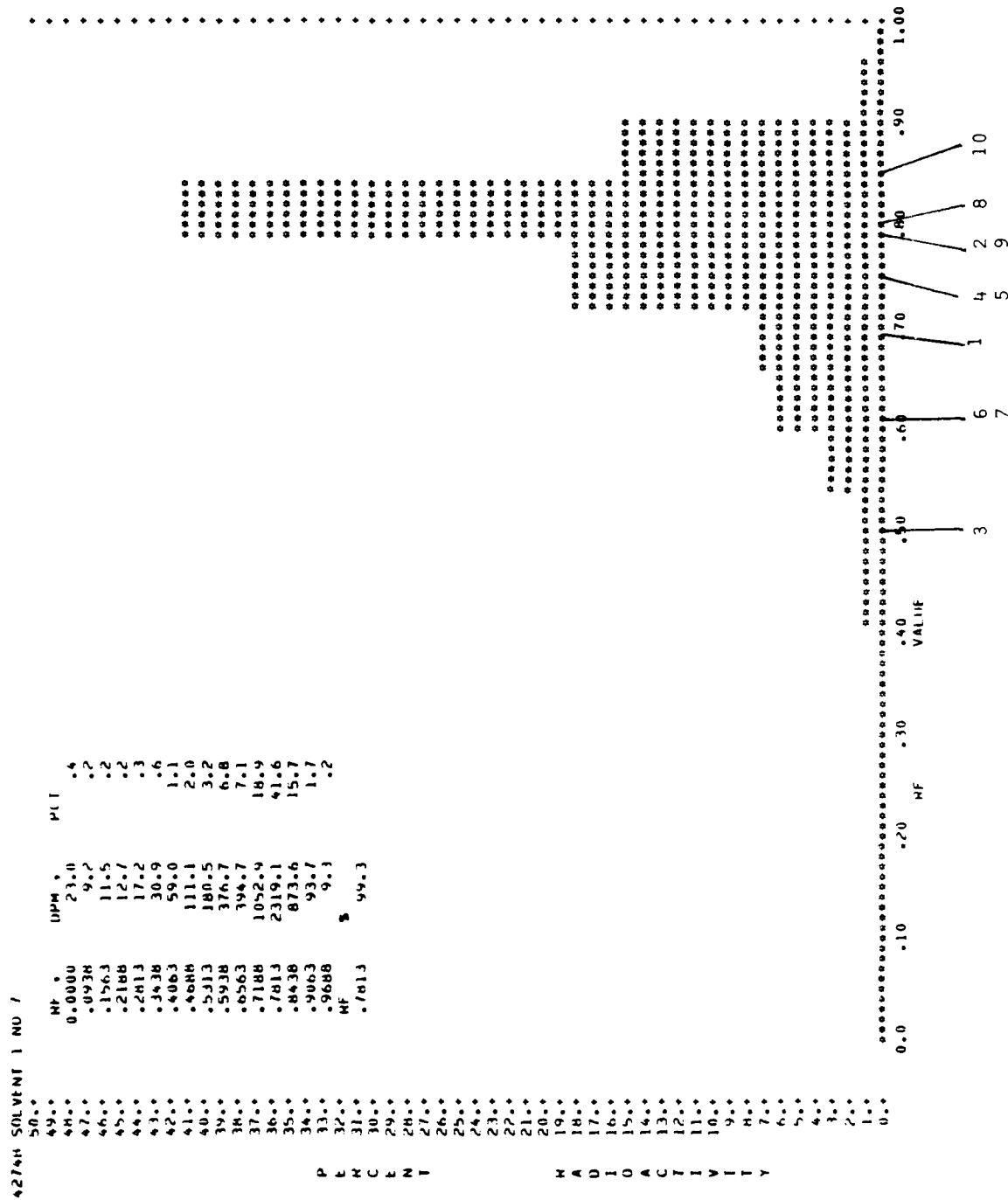


Figure 21-d-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX



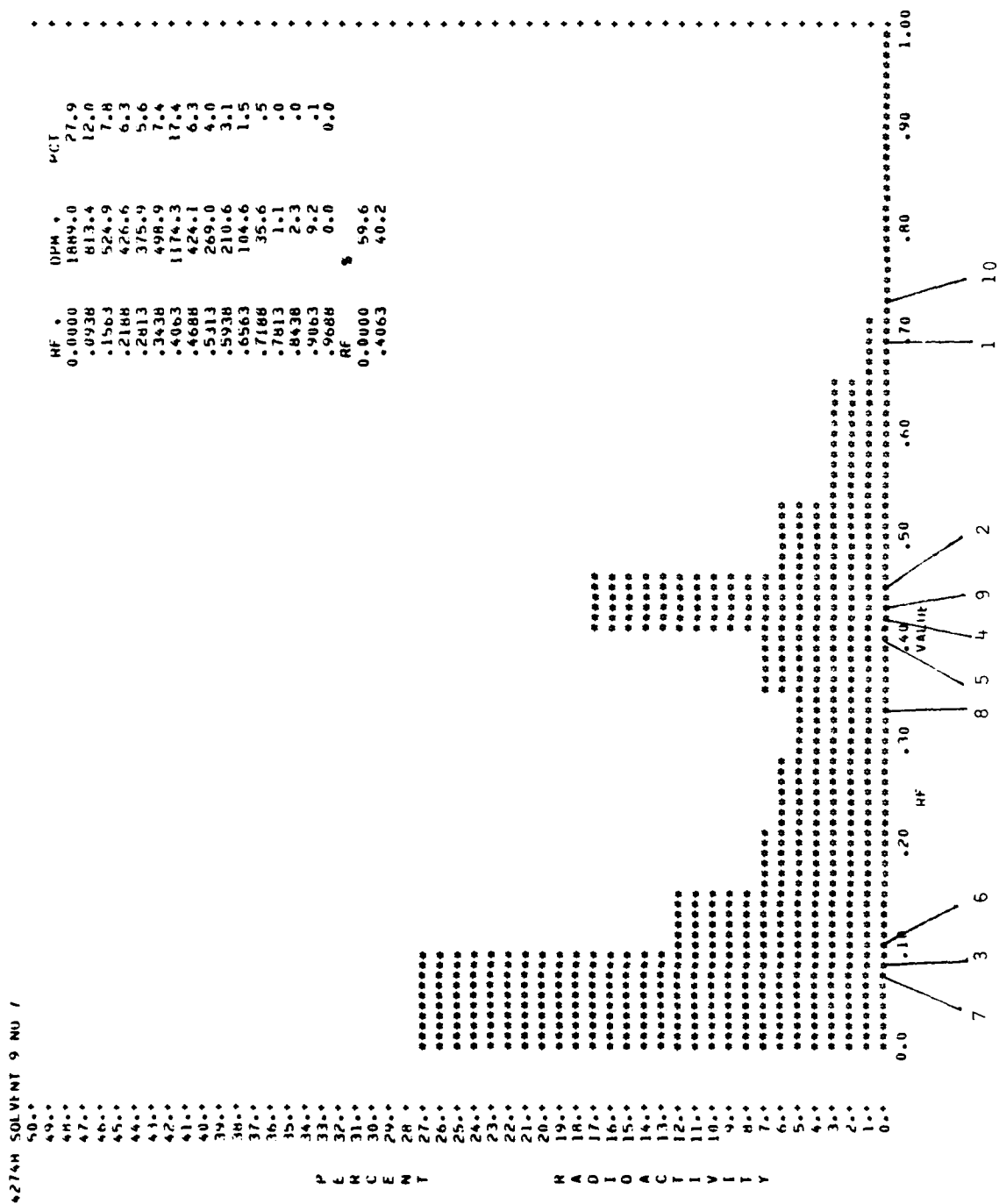


Figure 21-e-IX: Oral Treatment, Incubation with Water, Solvent IX

42/48 SOLVENT 1 NO 4

50..	RF	DPM	RF
69..	0.0000	28.7	.2
68..	.0938	29.4	.2
67..	.1563	35.4	.2
66..	.2188	43.7	.3
65..	.2813	48.2	.3
64..	.3438	105.5	.7
63..	.4063	216.1	1.4
62..	.4688	403.7	2.6
61..	.5313	667.4	4.4
60..	.5938	1176.9	7.7
59..	.6563	855.7	5.6
58..	.7188	2355.3	15.5
57..	.7813	6188.7	40.6
56..	.8438	2728.7	17.9
55..	.9063	337.6	2.2
54..	.9688	19.5	.1
53..	RF	\$	
52..	.5938	23.7	
51..	.7813	76.3	

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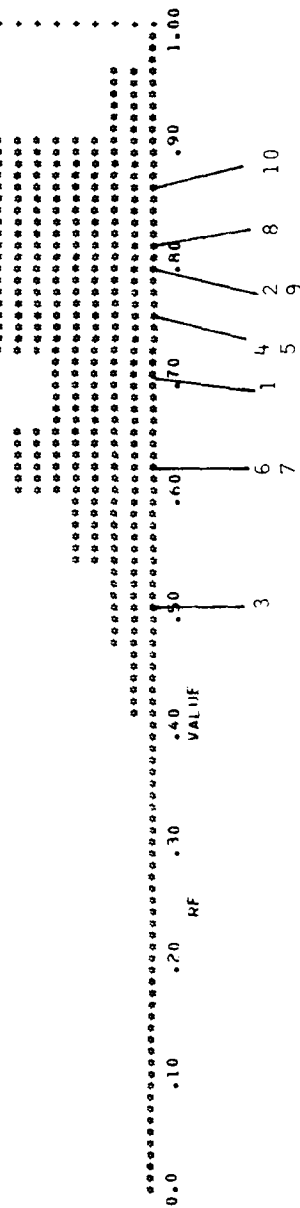


Figure 21-f-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

42/4H SOLVENT 9 NO 8

RF	DPM	MCT
0.0000	6371.5	30.1
.0936	2304.6	13.1
.1563	1998.9	11.3
.2188	1011.6	5.7
.2813	902.3	5.1
.3438	864.7	4.9
.4063	2619.5	14.9
.4688	643.7	3.7
.5313	383.7	2.2
.5938	241.4	1.4
.6563	205.7	1.2
.7188	67.7	.4
.7813	17.2	.1
.8438	0.0	0.0
.9063	0.0	0.0
.9688	0.0	0.0
RF		
0.0000	76.3	
.4063	23.7	

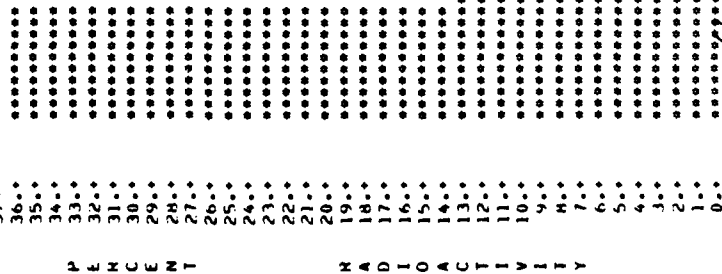


Figure 21-f-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

50.	44.	47.	46.	45.	44.	42.	41.	40.	39.	38.	37.	36.	35.	34.	33.	32.	31.	30.	29.	28.	27.	26.	25.	24.	23.	22.	21.	20.	19.	18.	17.	16.	15.	14.	13.	12.	11.	10.	9.	8.	7.	6.	5.	4.	3.	2.	1.	0.
										P E R C E N T										W A D I U O A C C T I V I T Y																												

HF *	DPM *	P.C.T.
.0000	20.9	.3
.0938	1.1	0
1.563	2.3	0
.2184	9.3	1
.2413	11.6	2
.3438	26.7	4
.4063	23.3	3
.4688	67.6	9
.5313	239.1	32
.5438	2400.9	31.8
.6562	373.3	4.9
.7188	2657.2	35.2
.7813	1281.8	16.5
.8438	376.5	5.0
.9063	81.6	1.1
.9688	9.3	.1
HF	%	
.5238	40.8	
.7188	57.9	

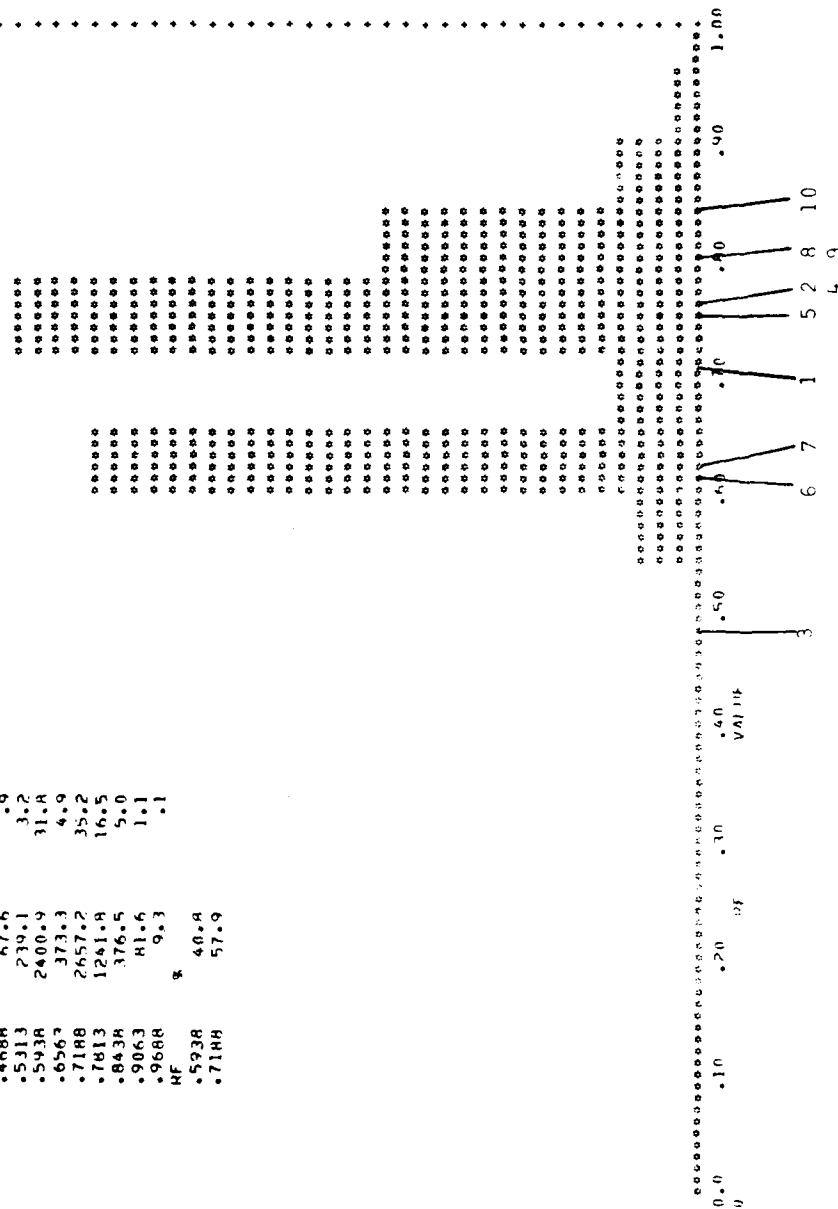


Figure 21-g-I: Dermal Application, Incubation with Water Solvent I

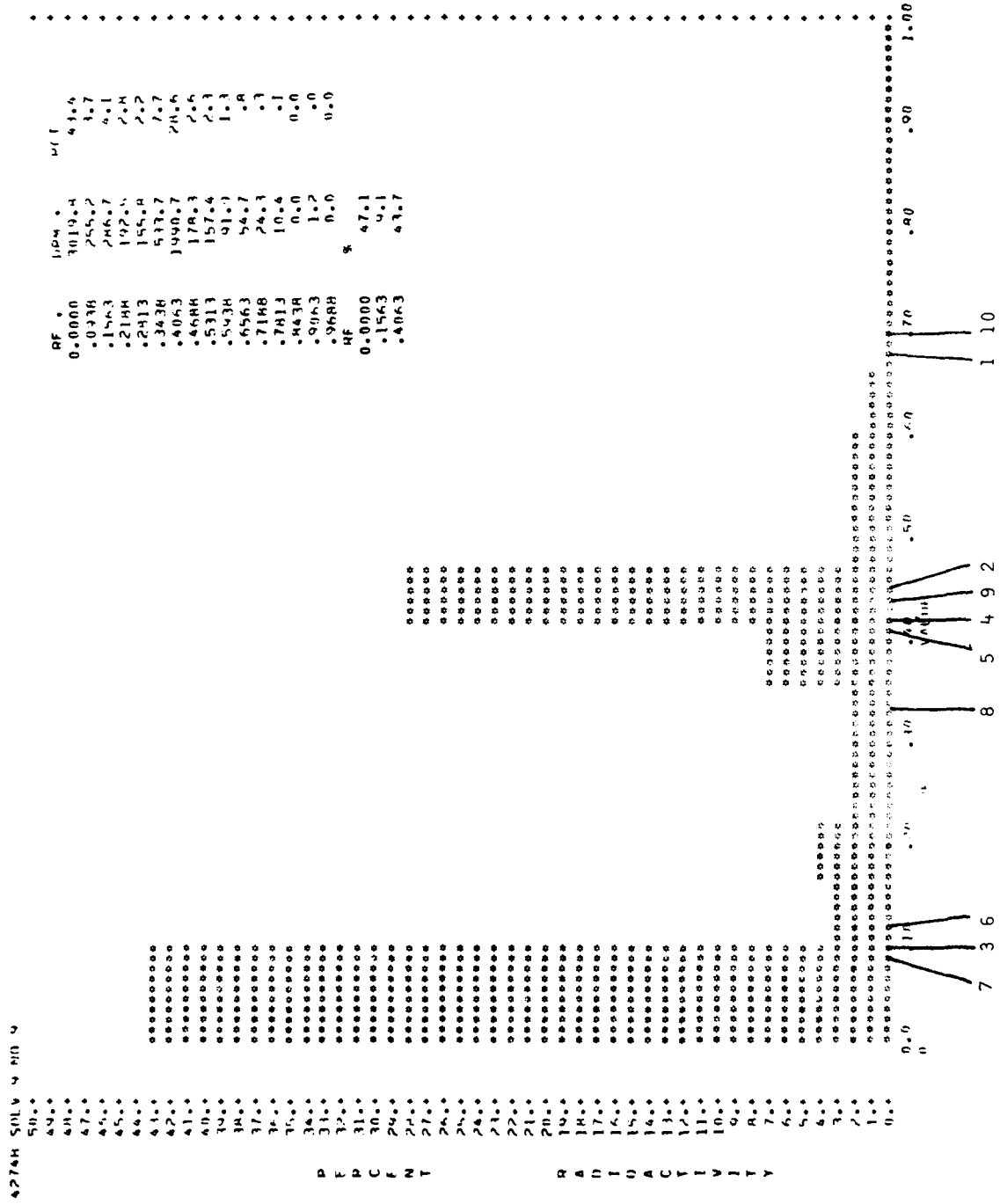


Figure 21-g-IX: Dermal Application, Incubation with Water, Solvent IX

42744 SOLV I NO 10

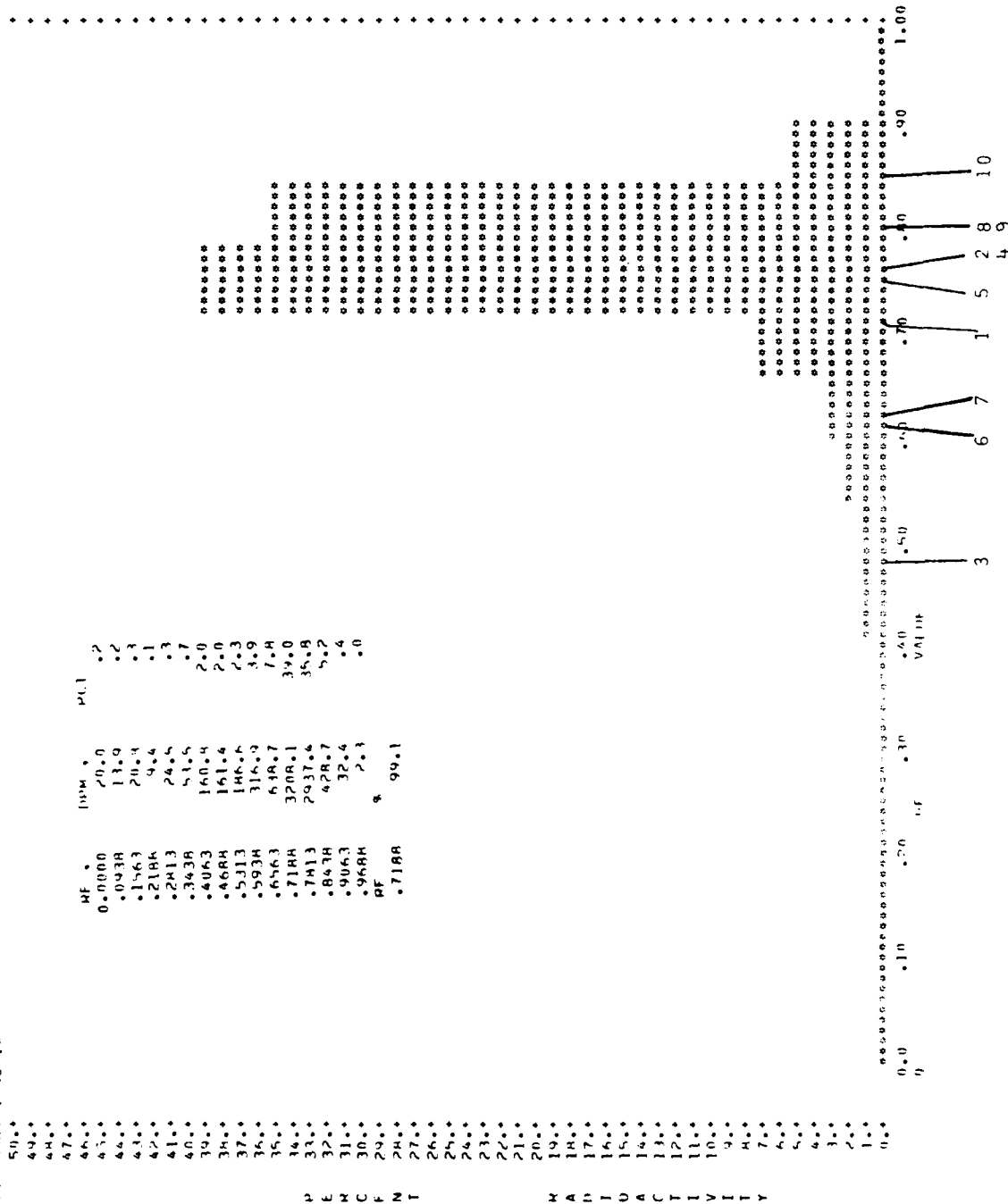


Figure 21-h-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

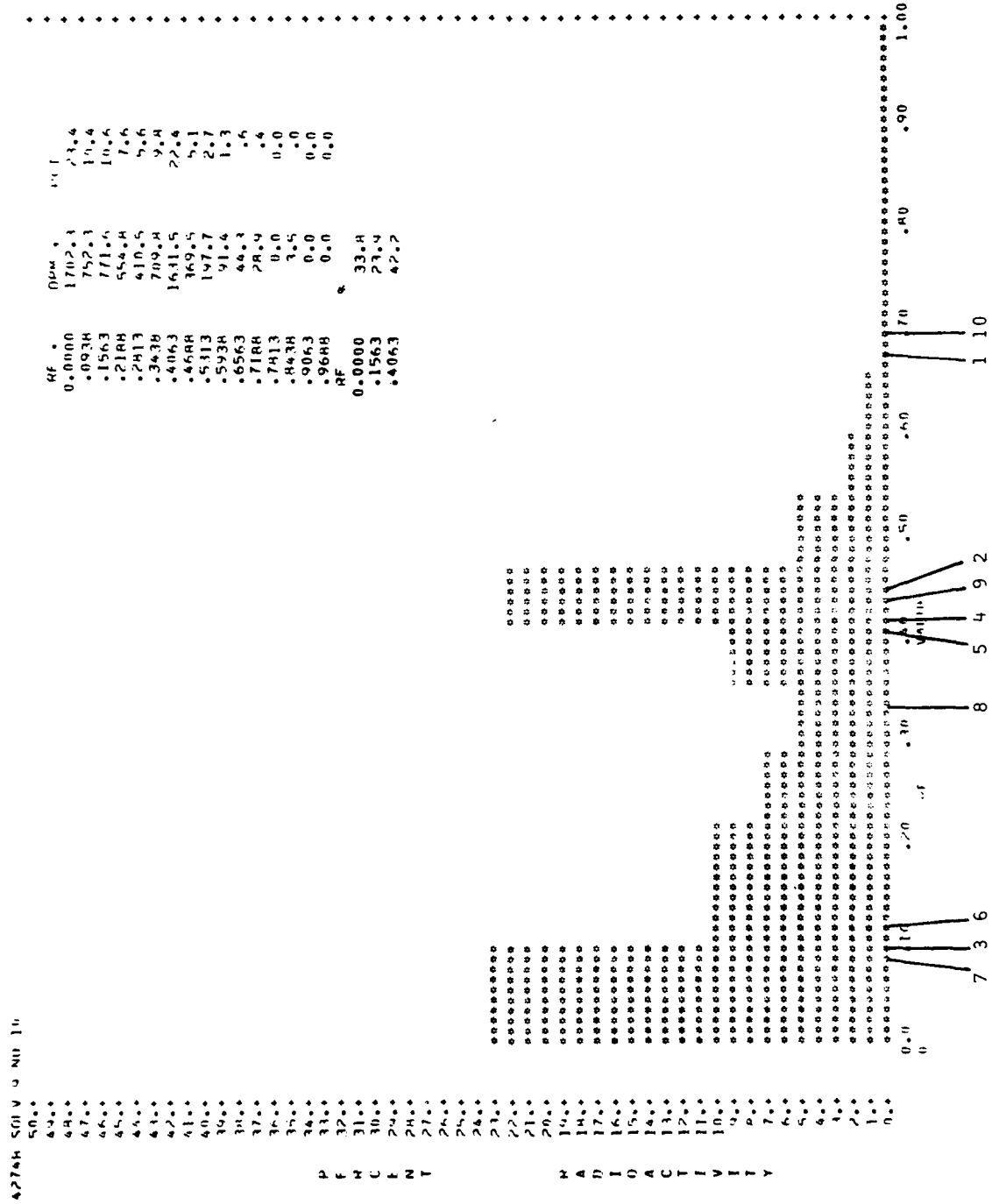


Figure 21-h-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

4274R SOLV 1 NO 15

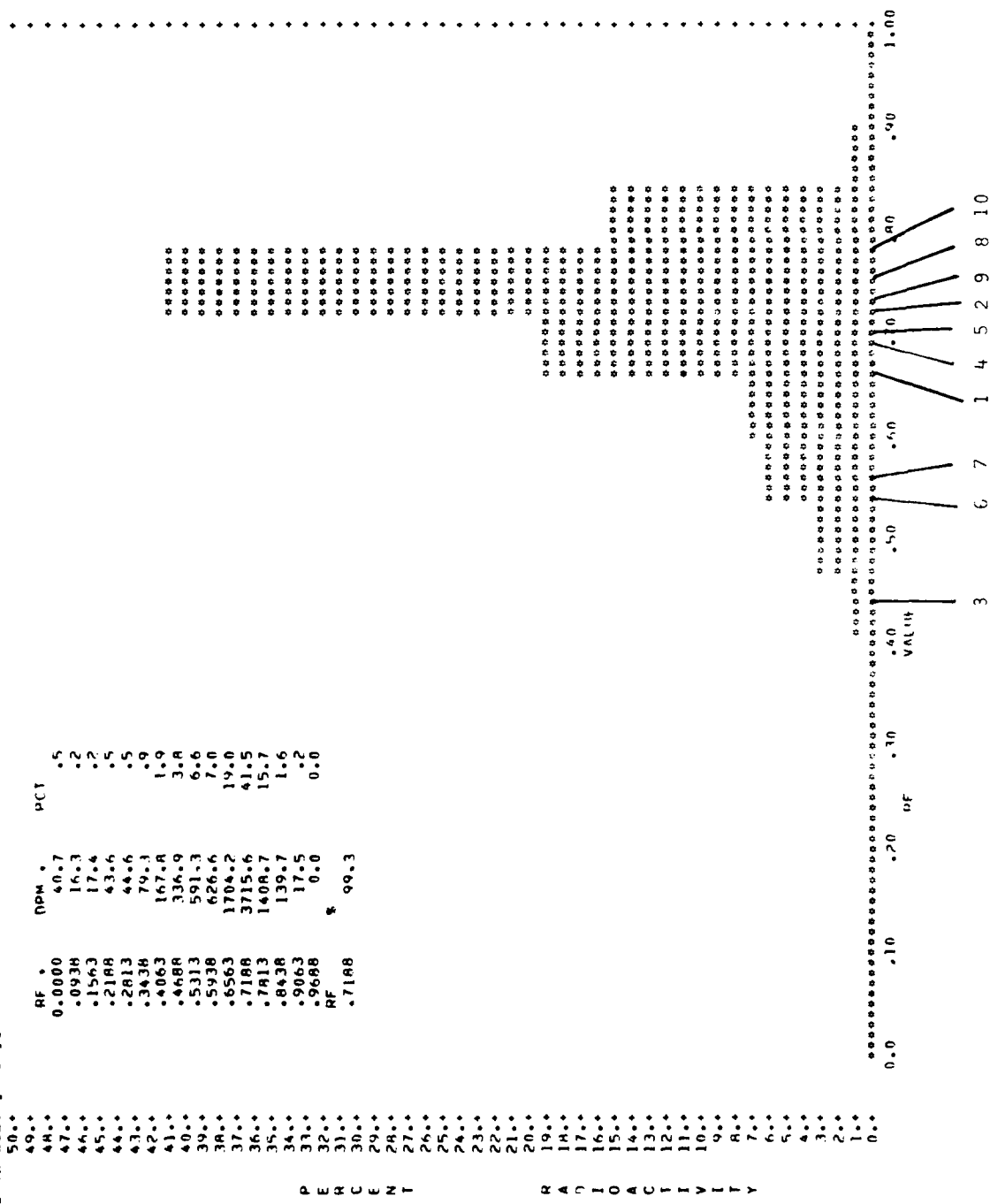


Figure 21-k-I: Oral Treatment, Incubation with Water, Solvent I

4274R SOLV 9 NO 15

50.0	RF	0.0000	DPM	2490.0	PCT	29.2
49.0		.0938		1096.3		12.9
48.0		.1563		721.1		8.5
47.0		.2188		560.2		6.6
46.0		.2813		576.3		6.8
45.0		.3438		1890.1		22.2
44.0		.4063		392.3		4.5
43.0		.4688		306.3		3.6
42.0		.5313		179.1		2.1
41.0		.5938		96.2		1.1
40.0		.6563		186.1		2.2
39.0		.7188		19.5		.2
38.0		.7813		7.0		.1
37.0		.8438		4.6		.1
36.0		.9063		1.2		.0
35.0		.9688		0.0		0.0
34.0						
33.0						
32.0						
31.0						
30.0						
29.0						
28.0						
27.0						
26.0						
25.0						
24.0						
23.0						
22.0						
21.0						
20.0						
19.0						
18.0						
17.0						
16.0						
15.0						
14.0						
13.0						
12.0						
11.0						
10.0						
9.0						
8.0						
7.0						
6.0						
5.0						
4.0						
3.0						
2.0						
1.0						
0.0						

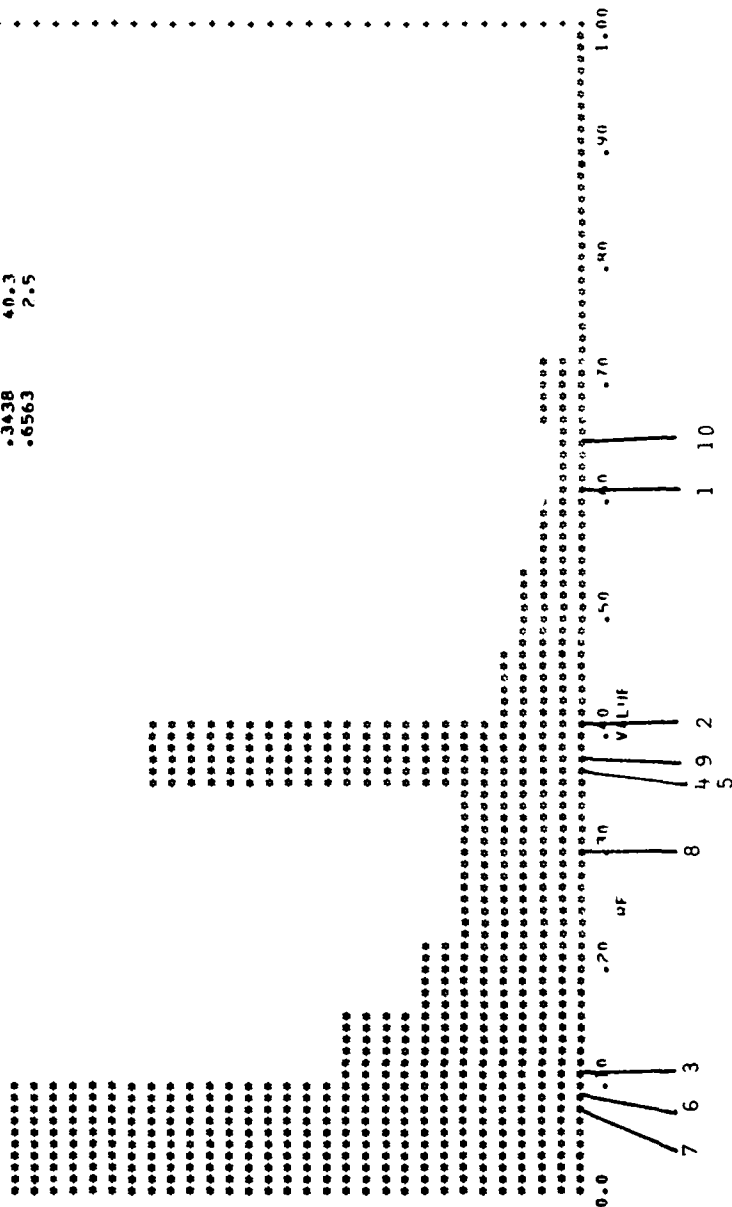


Figure 21-k-IX: Oral Treatment, Incubation with Water, Solvent IX

42748 SOLVENT 1 NO 6

HF	DPM	PUT
0.0000	9.2	1.5
.0938	9.2	1.5
.1563	4.6	.7
.2188	6.9	1.1
.2813	4.6	.7
.3438	5.7	.9
.4063	16.1	2.5
.4688	24.1	3.8
.5313	43.5	6.9
.5938	192.8	30.7
.6563	77.0	12.2
.7188	154.0	24.4
.7813	70.6	11.2
.8438	9.2	1.5
.9063	1.1	.2
.9688	2.3	.4
RF		
0.0000	3.5	
.5938	56.7	
.7188	37.0	

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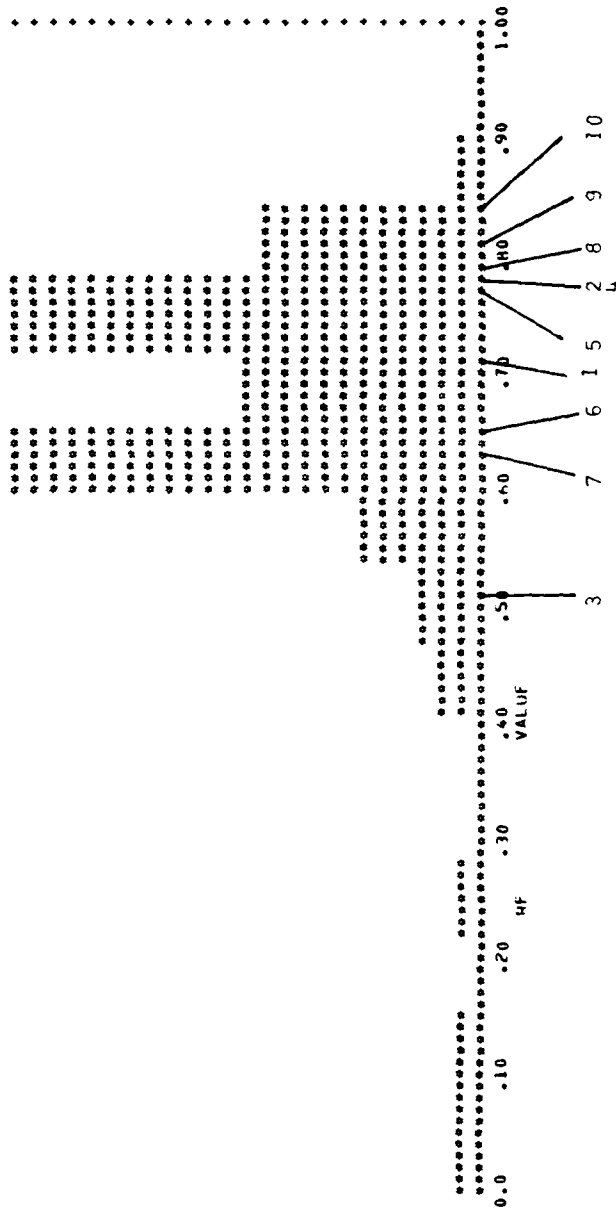


Figure 21-1-I: Oral Treatment, Incubation with β -Glucuronidase, Solvent I

42748 SOLVENT 9 NO 6

50.0

49.0

48.0

47.0

46.0

45.0

44.0

43.0

42.0

41.0

40.0

39.0

38.0

37.0

36.0

35.0

34.0

33.0

32.0

31.0

30.0

29.0

28.0

27.0

26.0

25.0

24.0

23.0

22.0

21.0

20.0

19.0

18.0

17.0

16.0

15.0

14.0

13.0

12.0

11.0

10.0

9.0

8.0

7.0

6.0

5.0

4.0

3.0

2.0

1.0

0.0

P E H C E N T

H A D I U A C T I V I T Y

HF .
0.0000
.0938
.1563
.2188
.2813
.3438
.4063
.4688
.5313
.5938
.6563
.7188
.7813
.8438
.9063
.9688
RF
0.0000
.3438
.5313

DPM .
339.4
57.5
57.3
32.2
23.1
78.6
50.5
13.9
41.3
13.9
3.4
8.0
2.3
0.0
6.9
69.7
19.3
7.8

PCT
46.6
7.9
7.9
4.4
3.2
10.8
6.9
1.9
5.7
1.9
.5
0.0
1.1
.3
0.0
.9

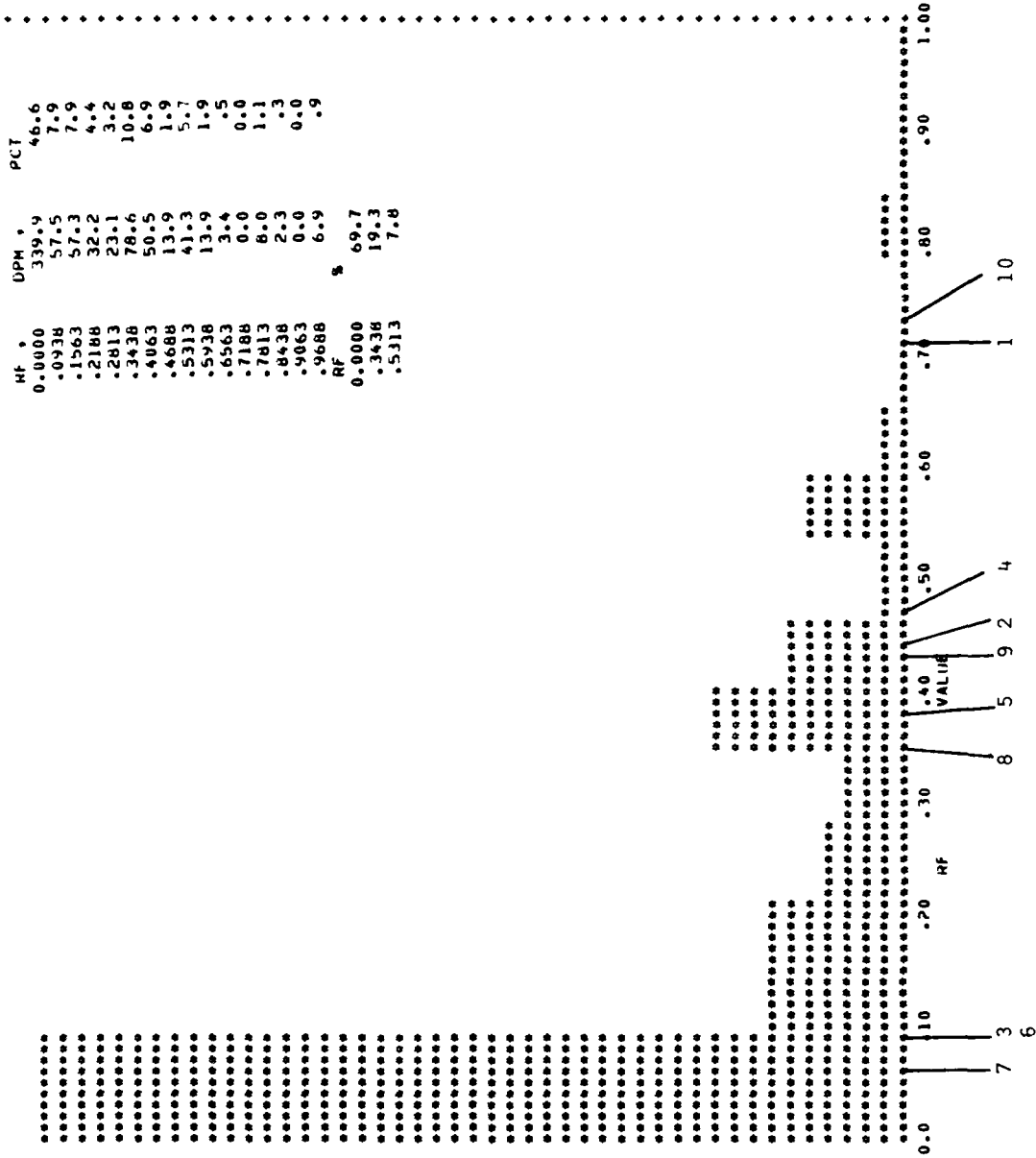


Figure 21-1-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

4274R SOLV I NO 13

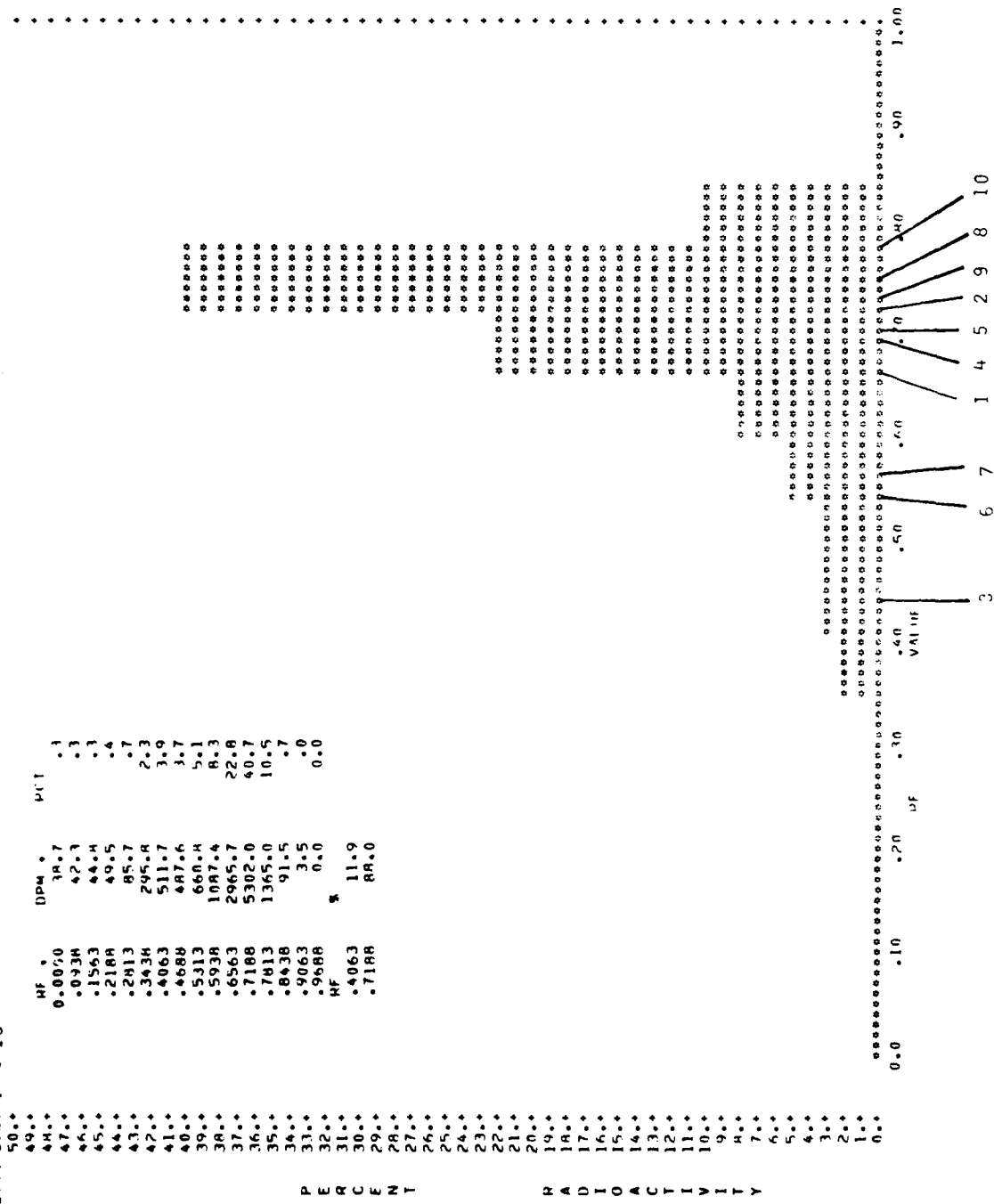


Figure 21-m-I: Dermal Application, Incubation with Water, Solvent I

42748 SOLV 9 NO 13

P	50.0	0.0000	10173.1	37.5
E	49.0	.0938	2667.4	9.8
M	48.0	.1563	2797.5	10.3
C	46.0	.2188	1629.0	6.0
E	45.0	.2413	1371.1	5.1
N	44.0	.3438	5715.1	21.1
T	43.0	.4063	934.7	3.4
	42.0	.4688	500.0	1.8
	41.0	.5313	394.4	1.5
	40.0	.5938	576.9	2.1
	39.0	.6563	72.3	.3
	38.0	.7188	21.0	.1
	37.0	.7813	11.7	.0
	36.0	.8438	245.3	.9
	35.0	.9063	0.0	0.0
	34.0	.9688	0.0	0.0
	33.0	RF		
	32.0	0.0000	47.4	
	31.0	.1563	21.4	
	30.0	.3438	27.8	
	29.0	.5938	2.5	

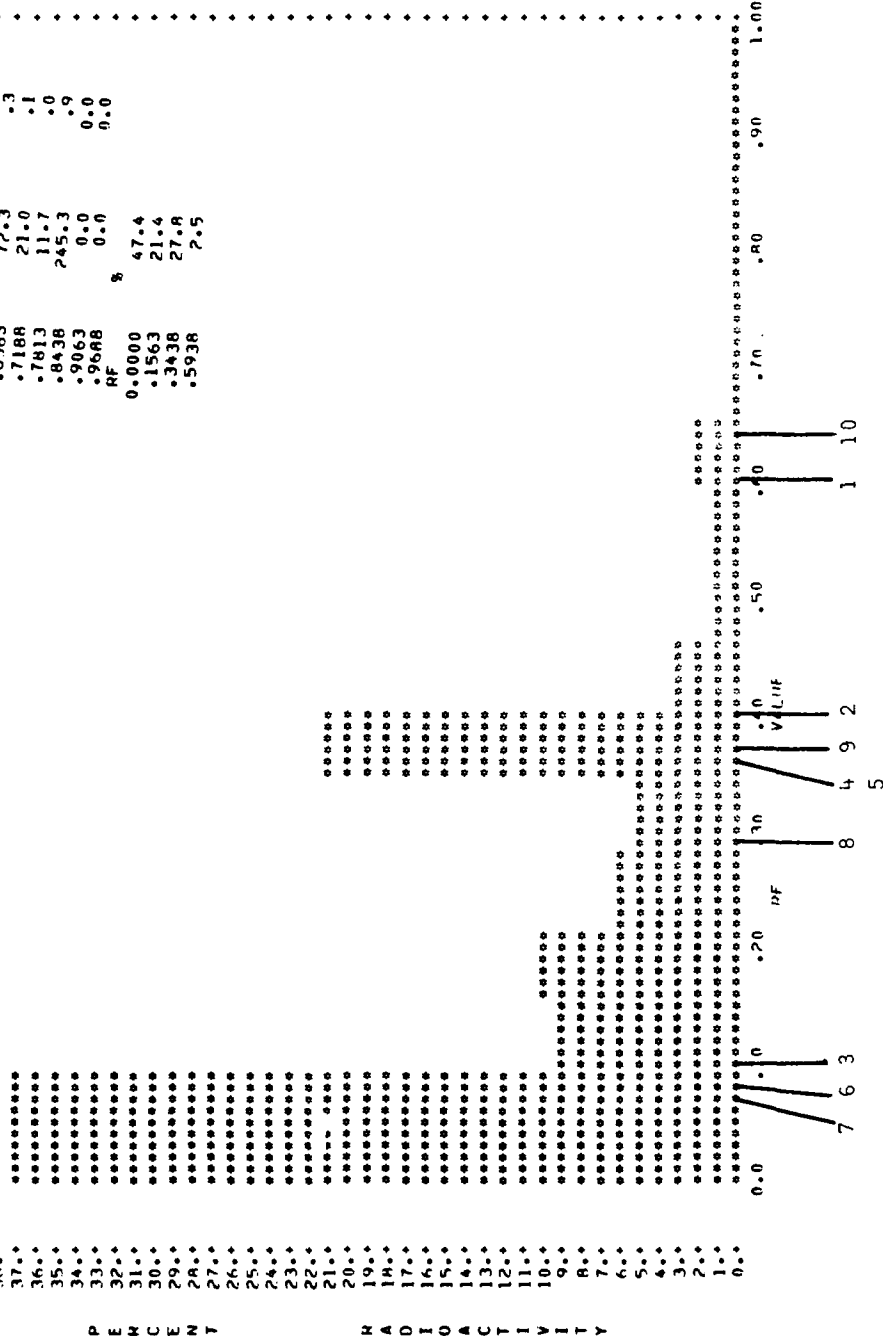


Figure 21-m-IX: Dermal Application, Incubation with Water, Solvent IX

42748 SOLVENT 1 NO 7

RF	DPM	RF	RF
50.0	0.0000	0.0000	0.6
49.0	19.5	0.2	0.2
48.0	6.9	0.4	0.4
47.0	13.8	0.4	0.4
46.0	13.8	0.5	0.5
45.0	21.88	1.1	1.1
44.0	28.13	1.7	1.7
43.0	34.38	1.7	1.7
42.0	40.63	44.0	44.0
41.0	46.88	12.0	12.0
40.0	53.13	15.0	15.0
39.0	59.38	18.4	18.4
38.0	65.63	3.3	3.3
37.0	71.88	0.0	0.0
36.0	78.13	0.1	0.1
35.0	84.38		
34.0	90.63		
33.0	96.88		
32.0			
31.0			
30.0			
29.0			
28.0			
27.0			
26.0			
25.0			
24.0			
23.0			
22.0			
21.0			
20.0			
19.0			
18.0			
17.0			
16.0			
15.0			
14.0			
13.0			
12.0			
11.0			
10.0			
9.0			
8.0			
7.0			
6.0			
5.0			
4.0			
3.0			
2.0			
1.0			
0.0			

P E R C E N T

H A U I O A C T I V I T Y

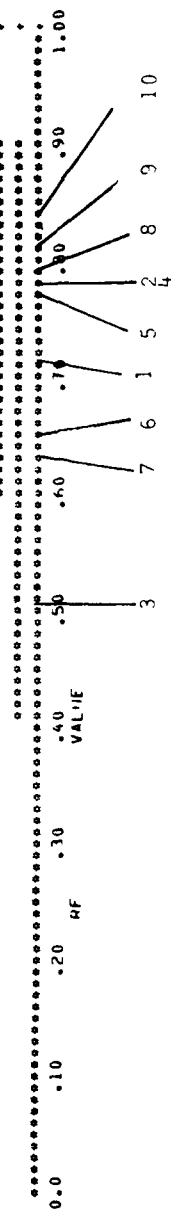


Figure 21-n-I: Dermal Application, Incubation with β -Glucuronidase, Solvent I

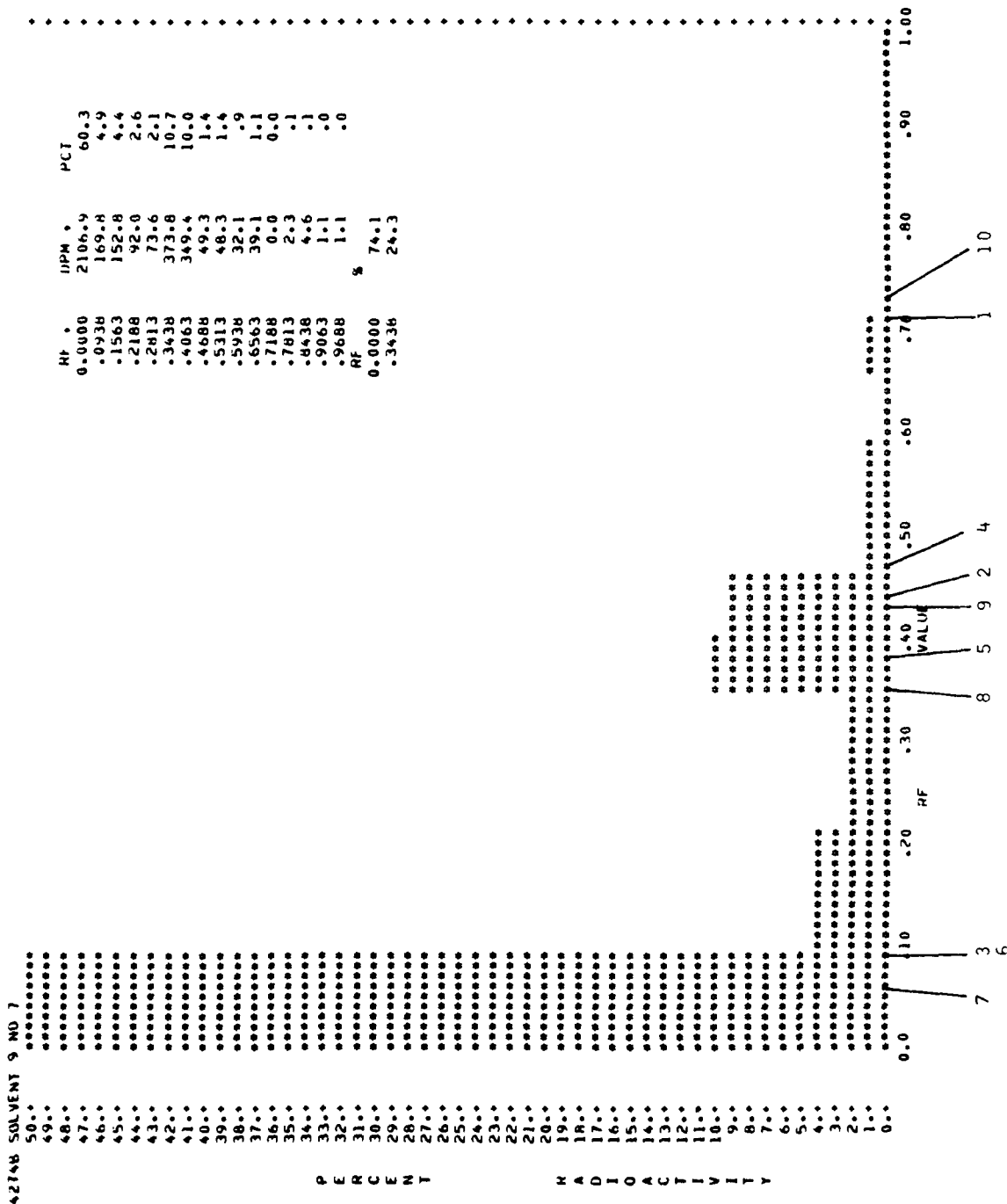


Figure 21-n-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

SOLVENT 1 NO 14

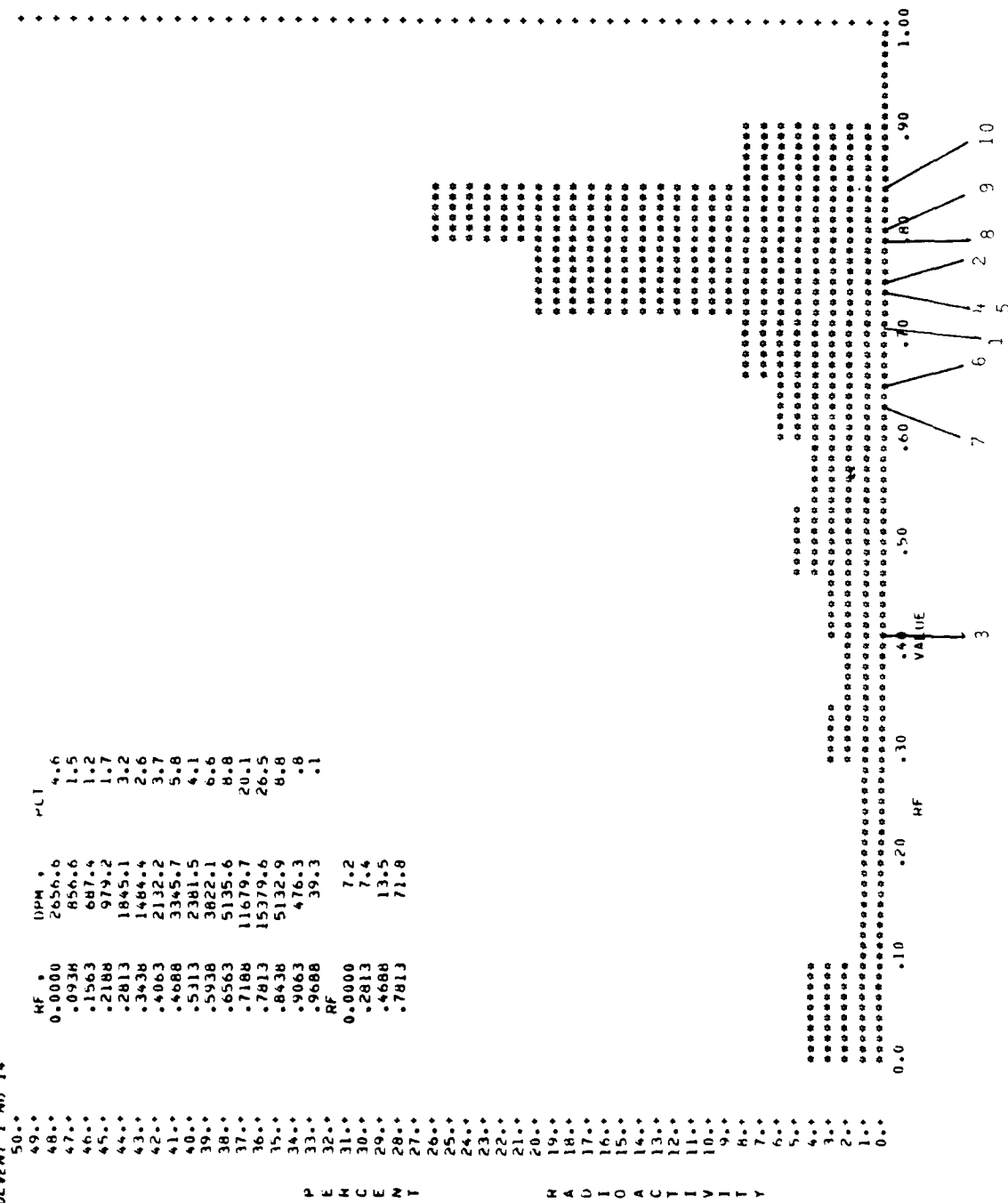


Figure 21-o-1: Oral Treatment, Incubation with β -Glucuronidase, Solvent 1

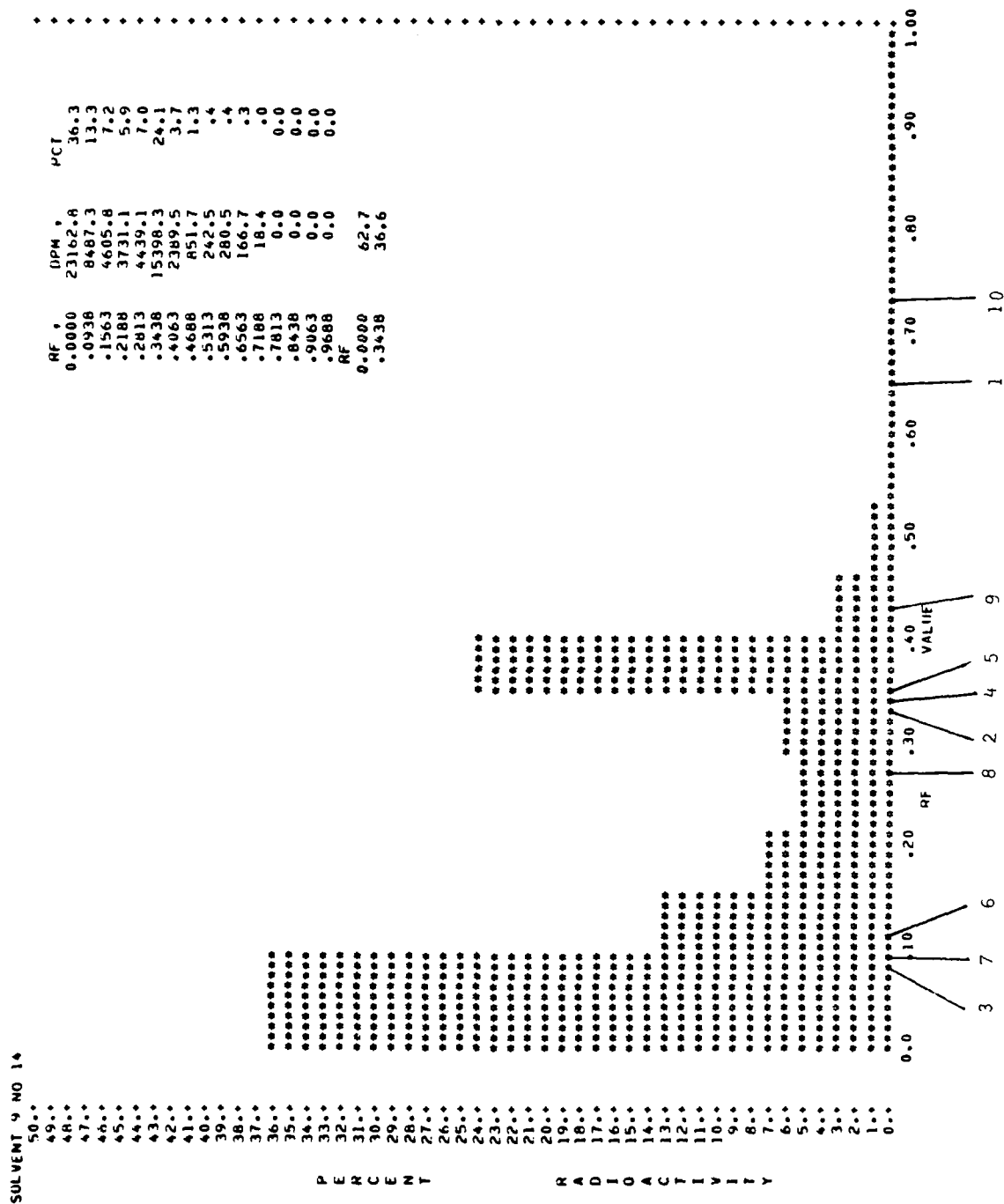


Figure 21-o-IX: Oral Treatment, Incubation with β -Glucuronidase, Solvent IX

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SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM OF 2,4,6-ETC(U)
JUN 81 A M EL-HAWARI, J R HODGSON

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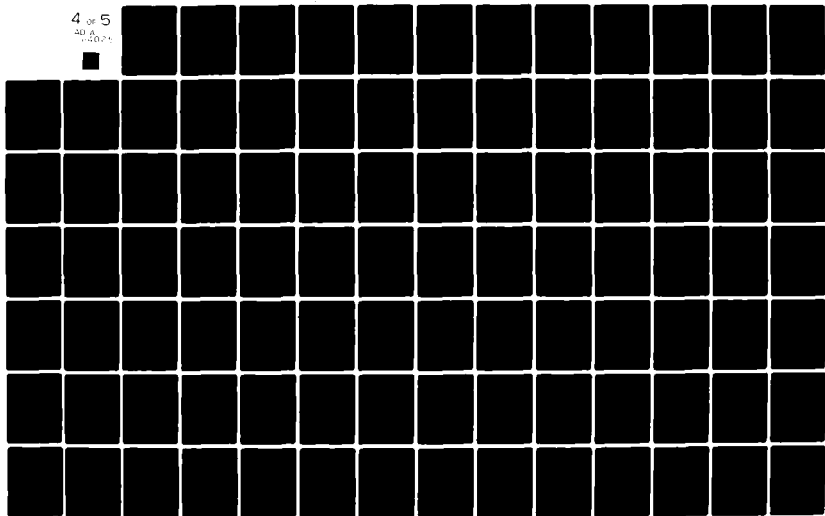
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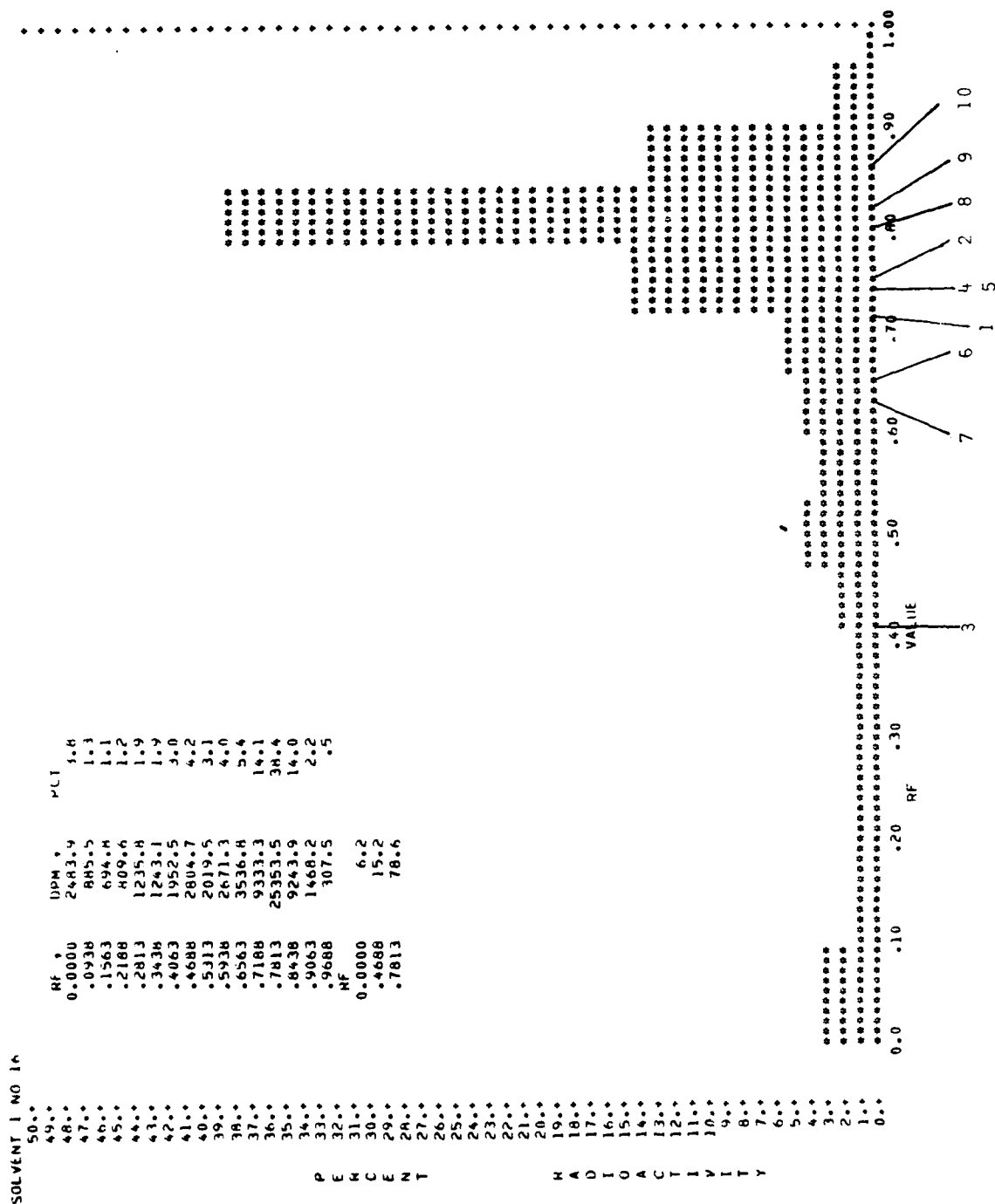


Figure 21-p-I: Dermal Application, Incubation with 8-Glucuronidase, Solvent I

RF	DPM	PCI
0.0000	19390.4	39.9
.0938	3157.3	6.5
.1563	2123.8	4.4
.2188	1963.0	4.0
.2813	2174.6	4.5
.3438	15031.0	30.9
.4063	3143.4	6.5
.4688	799.8	1.6
.5313	294.7	.6
.5938	332.6	.7
.6563	167.6	.3
.7188	25.5	.1
.7813	3.5	.0
.8438	0.0	0.0
.9063	1.1	.0
.9688	0.0	0.0
RF		
0.0000	54.0	
.3438	44.1	

Figure 21-p-IX: Dermal Application, Incubation with β -Glucuronidase, Solvent IX

Figure 22: TLC of Ethyl Acetate-Extractable Products Obtained from 24-Hr Urine of Male Dogs Treated Orally or Dermally with ^{14}C -TNT. Samples of urine were incubated with acetate buffer and β -glucuronidase before extraction with ethyl acetate. Incubation with acetate buffer and water served as control. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 22 follows

427416 SOLVENT 1 NO 11

PPM	HF	PLI
49.0	0.0000	.5
48.0	.0938	.3
47.0	.1563	.4
46.0	.2188	.6
45.0	.2813	.7
44.0	.3438	1.1
43.0	.4063	1.9
42.0	.4688	6.2
41.0	.5313	4.2
40.0	.5938	5.4
39.0	.6563	8.2
38.0	.7188	20.0
37.0	.7813	34.7
36.0	.8438	14.3
35.0	.9063	1.4
34.0	.9688	.2
33.0	HF	
32.0		
31.0		
30.0		
29.0		
28.0		
27.0		
26.0		
25.0		
24.0		
23.0		
22.0		
21.0		
20.0		
19.0		
18.0		
17.0		
16.0		
15.0		
14.0		
13.0		
12.0		
11.0		
10.0		
9.0		
8.0		
7.0		
6.0		
5.0		
4.0		
3.0		
2.0		
1.0		
0.0		

P E H C E N T

H A U O A C T I V I T Y

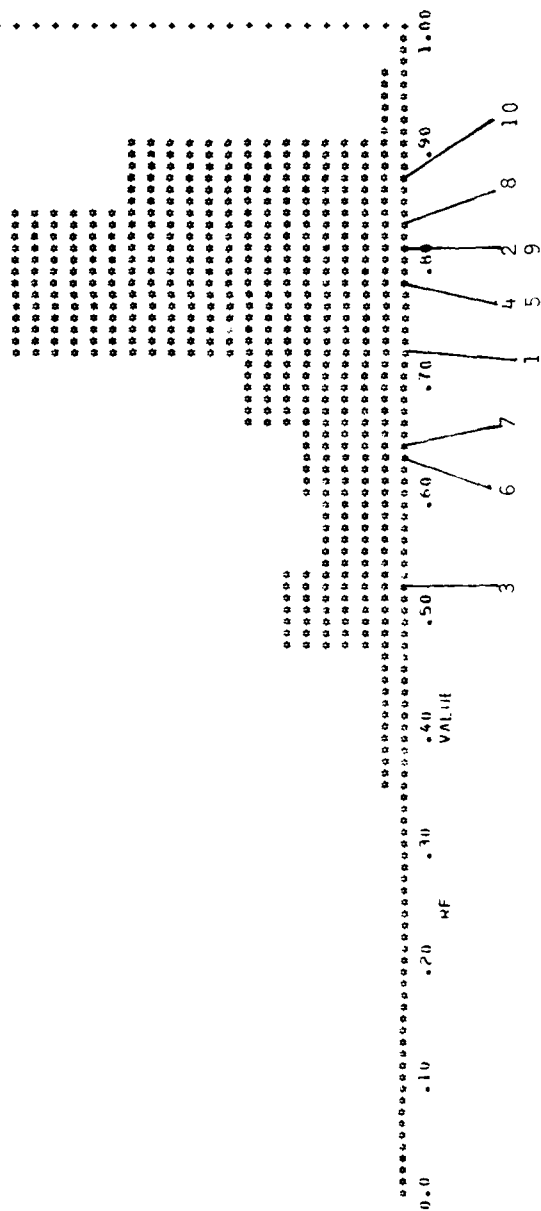


Figure 22-a-I: Oral Treatment, Incubation with Water, Solvent I.

42748 SOLVENT 9 NO 11

50..	RF	IPM	PCT
49..	0.0000	8492.0	24.7
48..	.0938	4468.6	13.0
47..	.1563	2452.5	7.1
46..	.2188	2518.3	7.3
45..	.2813	2219.9	6.5
44..	.3438	2537.6	7.4
43..	.4063	4462.1	13.0
42..	.4688	2260.9	6.6
41..	.5313	3005.8	8.8
40..	.5938	1198.8	3.5
39..	.6563	440.5	1.3
38..	.7188	243.1	.7
37..	.7813	20.6	.1
36..	.8438	1.2	.0
35..	.9063	0.0	0.0
34..	.9688	0.0	0.0
33..	RF	S	
32..	0.0000	44.9	
31..	.2188	13.8	
30..	.4063	27.0	
29..	.5313	14.3	
28..			
27..			
26..			
25..			
24..			
23..			
22..			
21..			
20..			
19..			
18..			
17..			
16..			
15..			
14..			
13..			
12..			
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4..			
3..			
2..			
1..			
0..			

P E R C E N T

R A D I O A C T I V I T Y

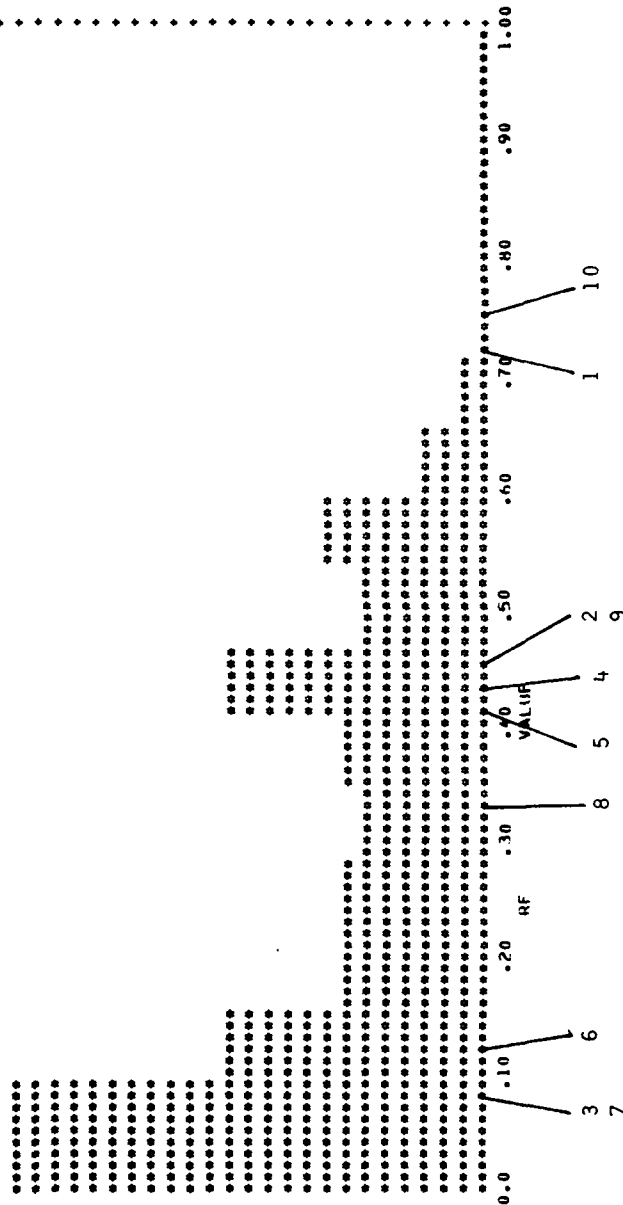


Figure 22-a-IX: Oral Treatment, Incubation with Water, Solvent IX.

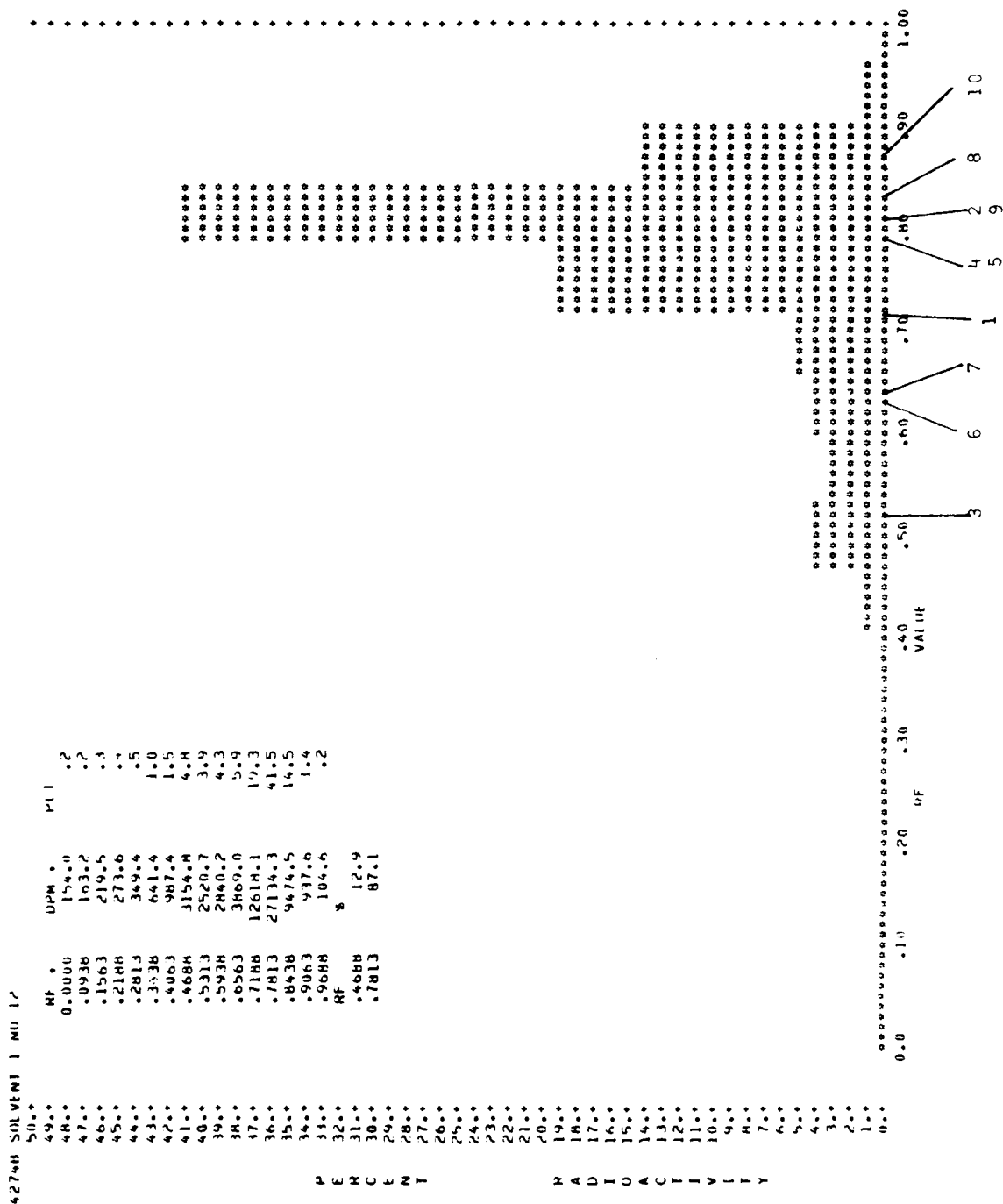


Figure 22-b-I: Oral Treatment, Incubation with B-glucuronidase, Solvent I.

4274H SOLVENT 9 MD 12

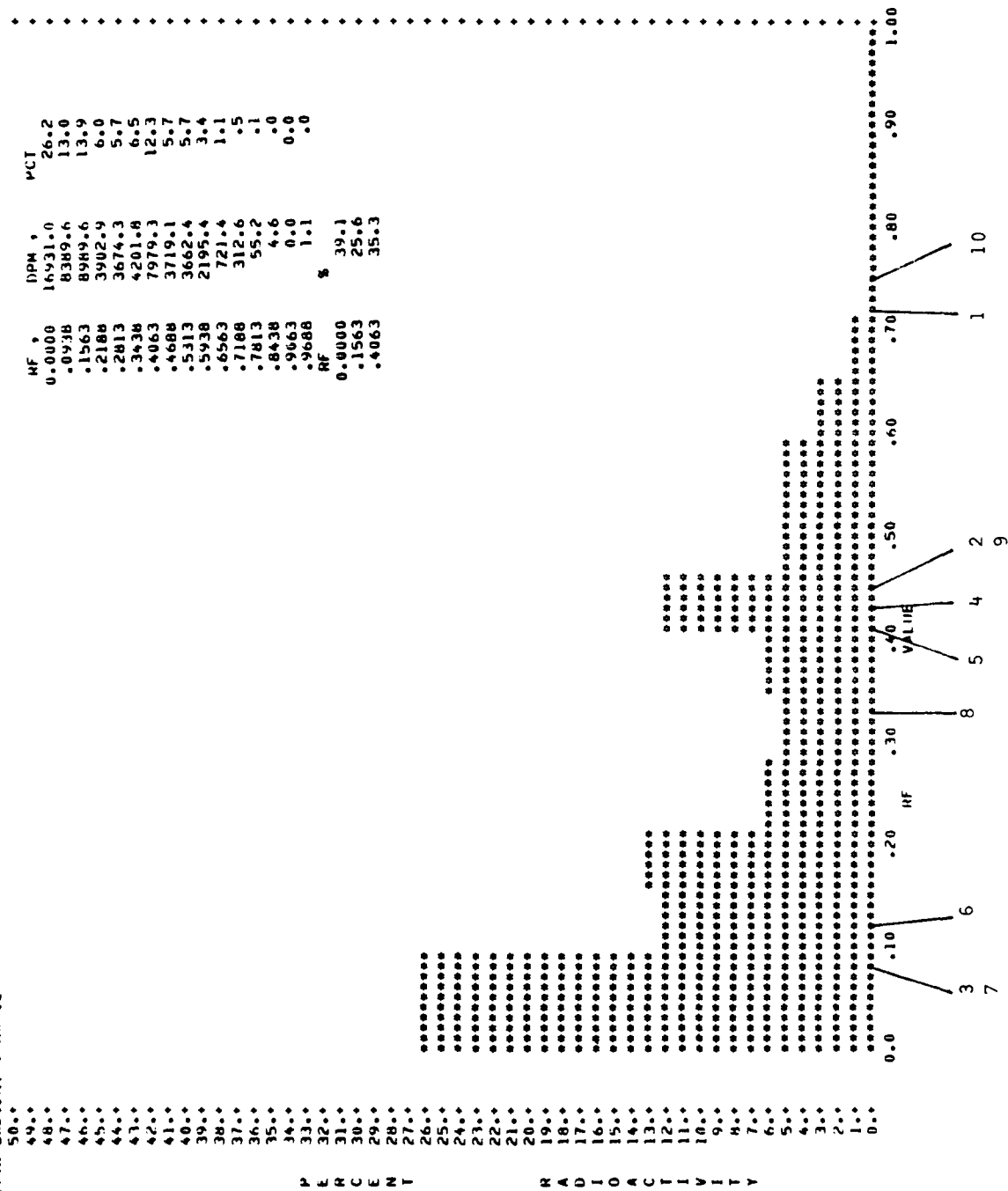


Figure 22-b-IX: Oral Treatment, Incubation with B-glucuronidase, Solvent IX.

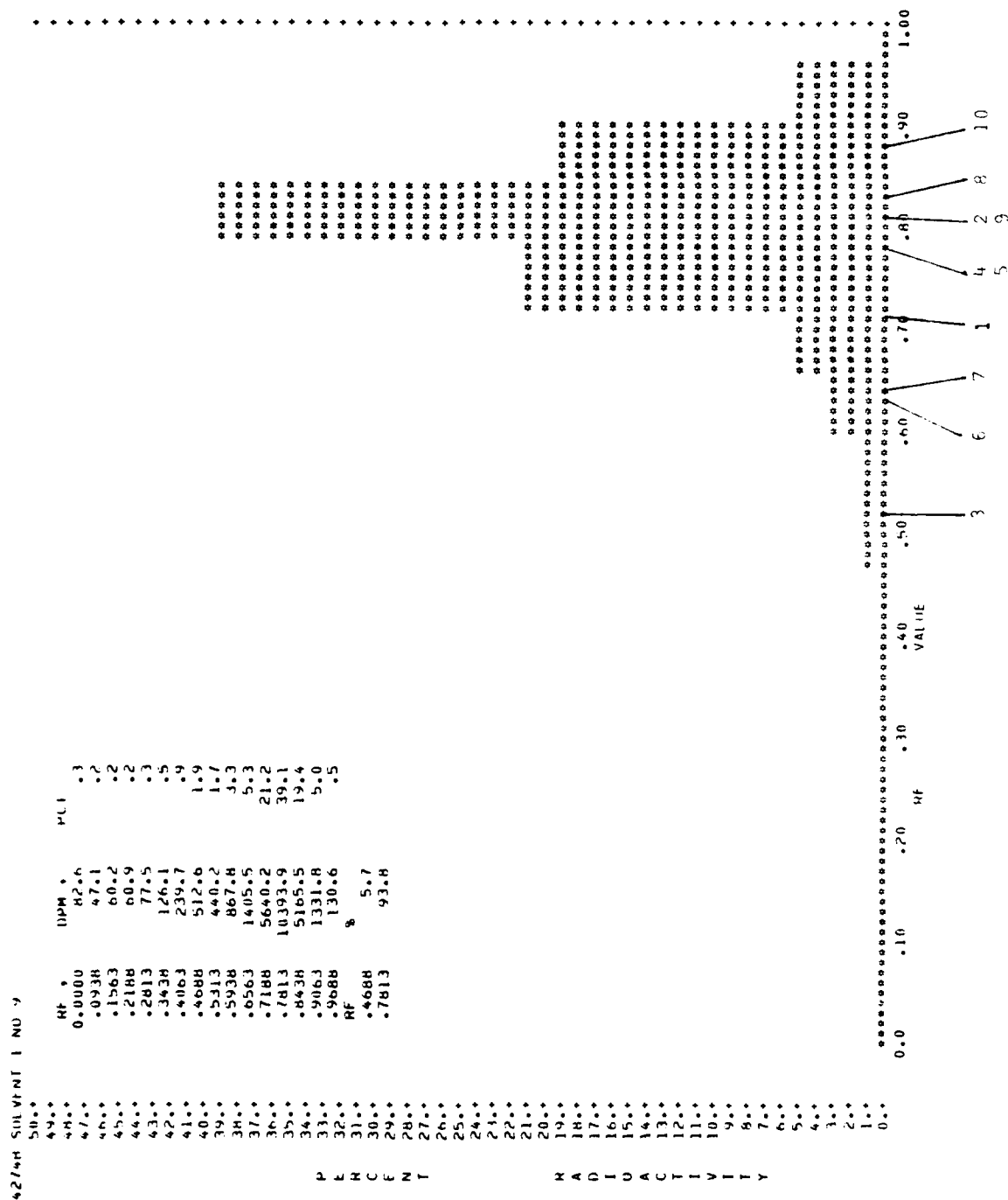


Figure 22-c-I: Dermal Application, Incubation with Water, Solvent I.

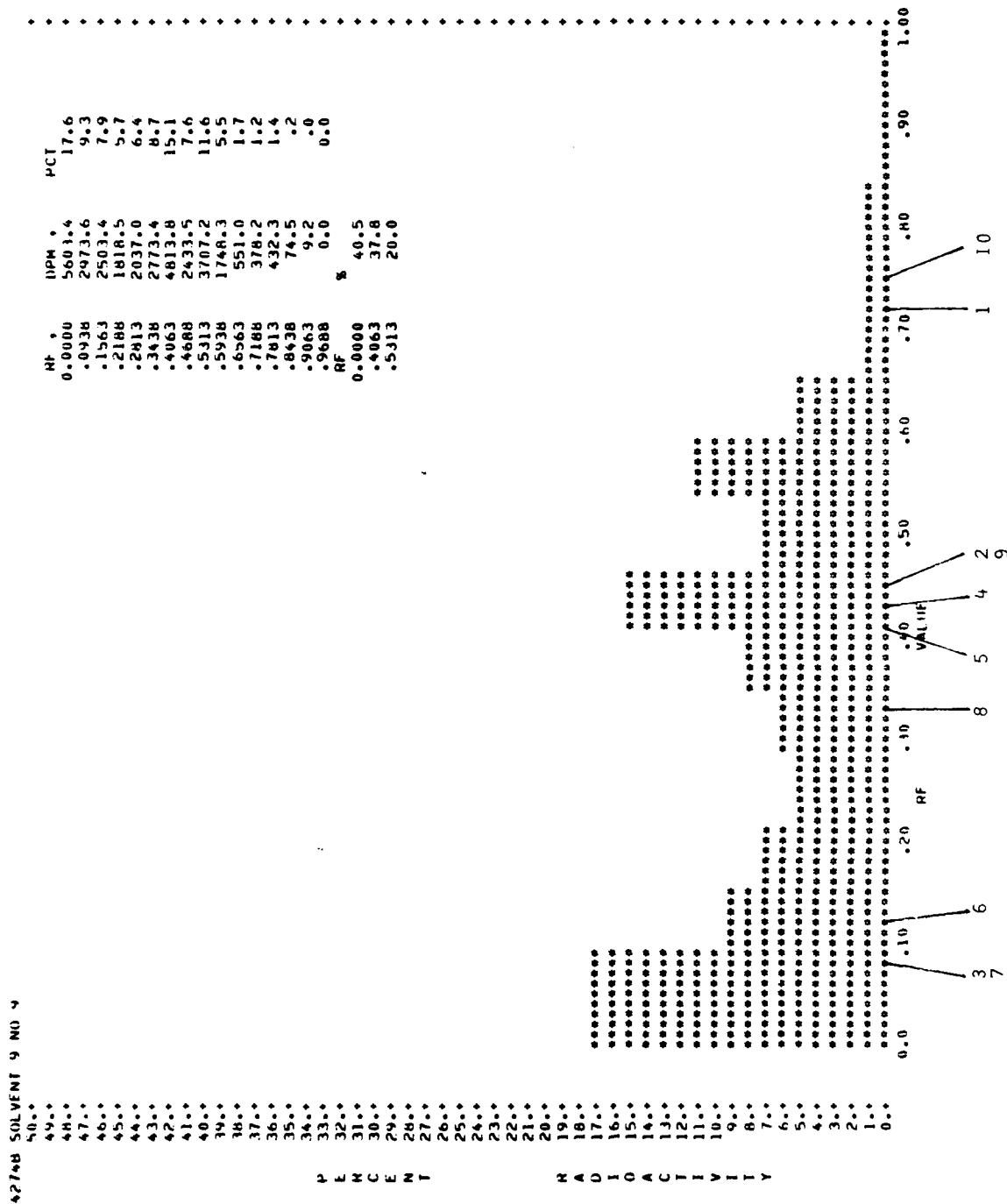


Figure 22-c-IX: Dermal Application, Incubation with Water, Solvent IX.

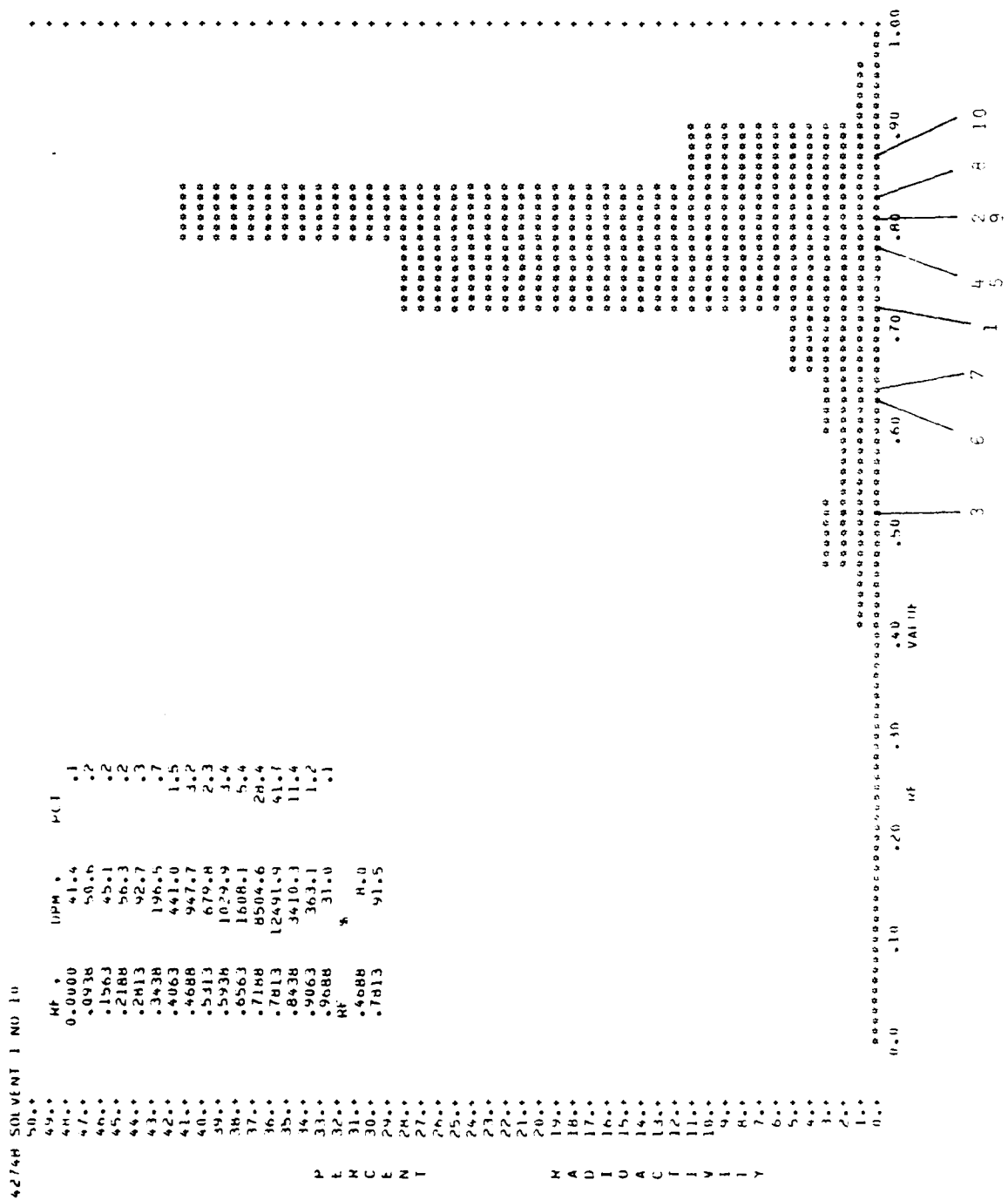


Figure 24-d-1: Dermal Application, Incubation with B-glucuronidase, Solvent 1.

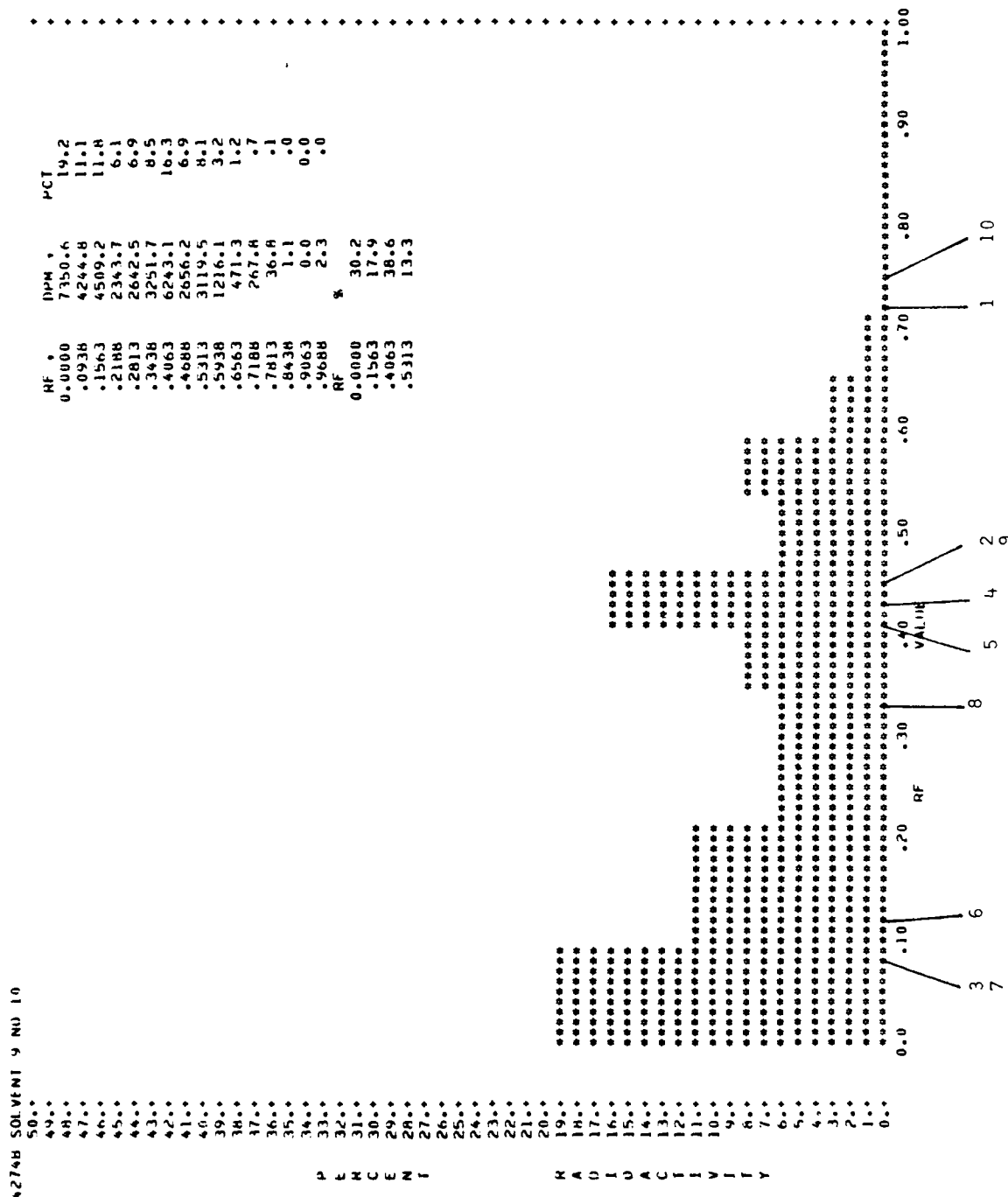


Figure 22-d-IX: Dermal Application, Incubation with B-glucuronidase, Solvent IX.

SOLVENT 1 NO 1

50.0	HF	DPM	PCT
49.0	0.0000	3489.0	3.4
48.0	.0938	2161.8	2.1
47.0	.1563	1342.9	1.3
46.0	.2188	1819.7	1.8
45.0	.2813	2320.7	2.2
44.0	.3438	2238.2	2.2
43.0	.4063	3176.9	3.1
42.0	.4688	8650.7	8.6
41.0	.5313	5668.2	5.5
40.0	.5938	7704.0	7.5
39.0	.6563	10334.5	10.0
38.0	.7188	21126.8	20.5
37.0	.7813	28939.8	28.0
36.0	.8438	3654.0	3.5
35.0	.9063	439.1	.4
34.0	.9688	19.5	.0
33.0	RF		
32.0	0.0000	6.8	
31.0	.2813	6.2	
30.0	.4688	17.1	
29.0	.7813	69.9	

P E N T

H A D I O A C T I V

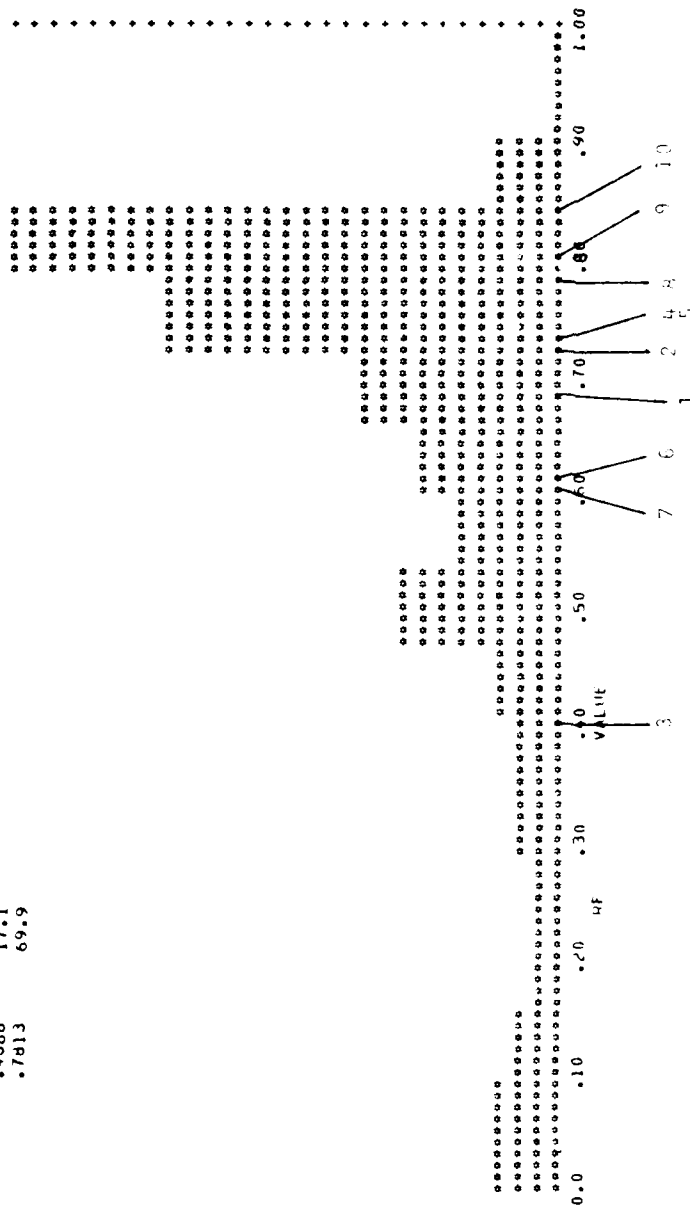


Figure 22-1-1: Oral Treatment, Incubation with Water, Solvent 1.

427-H JUNE 28 006 AND MAHIT EXTRACTIONS SOLVENT 9 NO 1

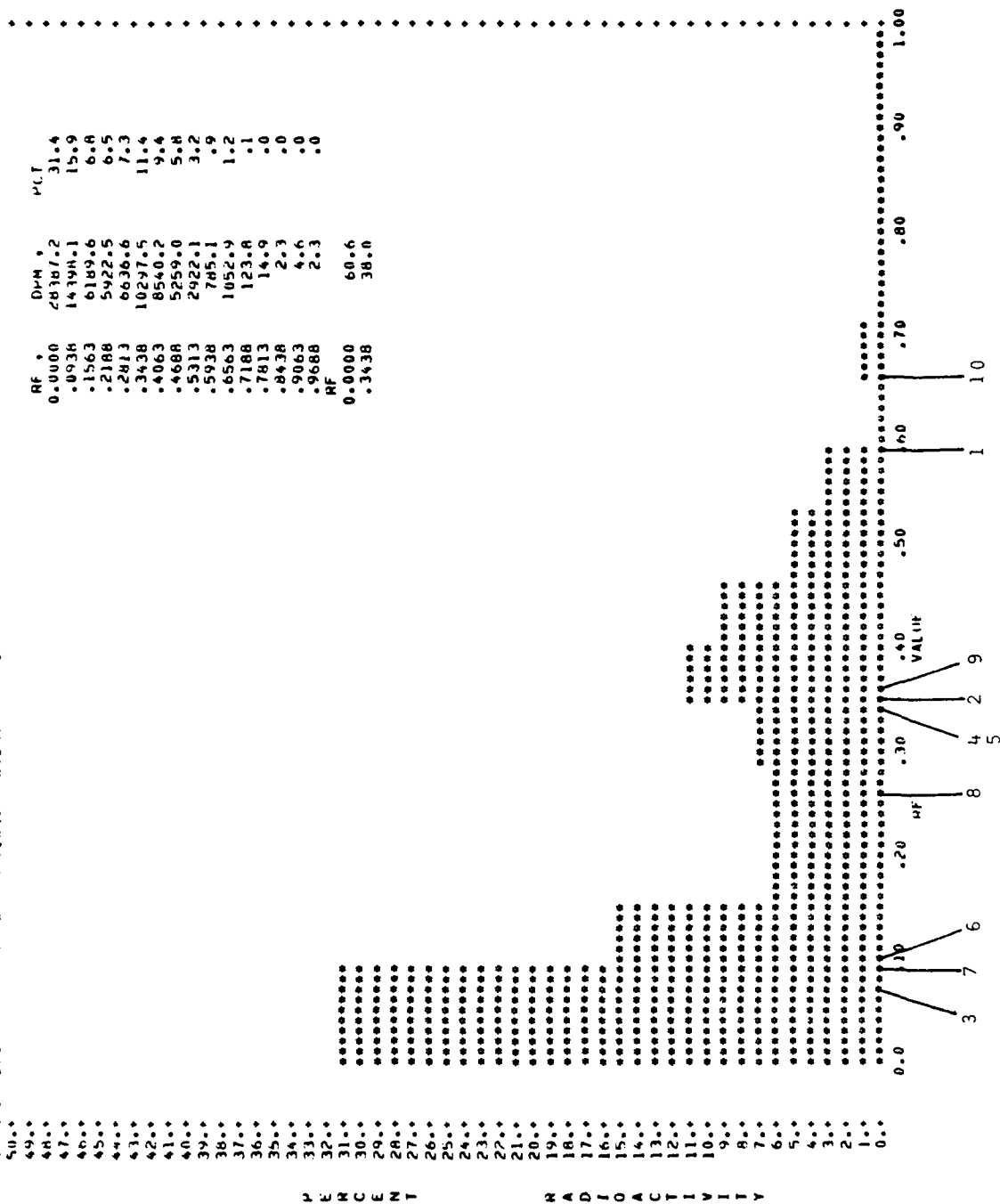


Figure 22-e-IX: Oral Treatment, Incubation with Water, Solvent IX.

SOLVENT 1 NO 2

50..	RF	DPM	MCI
49..	0.0000	1759.5	2.8
48..	.0938	850.9	1.4
47..	.1563	664.0	1.1
46..	.2188	733.3	1.2
45..	.2813	1225.3	1.9
44..	.3438	1125.3	1.8
43..	.4063	1340.3	2.1
42..	.4688	2995.4	4.8
41..	.5313	2395.6	3.8
40..	.5938	4863.6	7.7
39..	.6563	5359.3	8.5
38..	.7188	17286.0	27.4
37..	.7813	19711.8	31.3
36..	.8438	2330.3	3.7
35..	.9063	342.6	.5
34..	.9688	23.0	.0
33..	RF		
32..	0.0000	5.2	
31..	.2813	4.9	
30..	.4688	10.7	
29..	.7813	79.2	
28..			
27..			
26..			
25..			
24..			
23..			
22..			
21..			
20..			
19..			
18..			
17..			
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14..			
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7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E R C E N T

R A D I O A C T I V I T Y

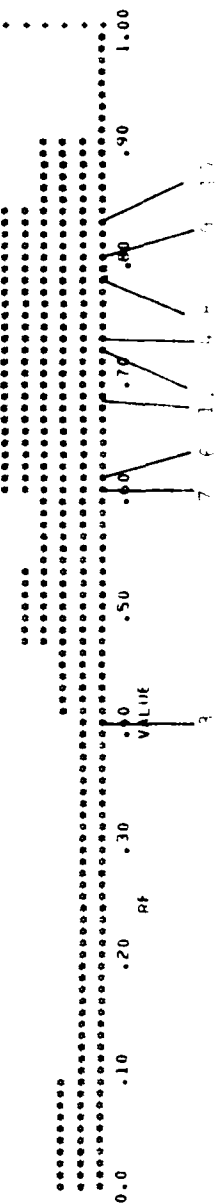


Figure 22-1-1: Oral Treatment, Incubation with B-galactosidase, Solvent 1.

SOLVENT 9 NO 2

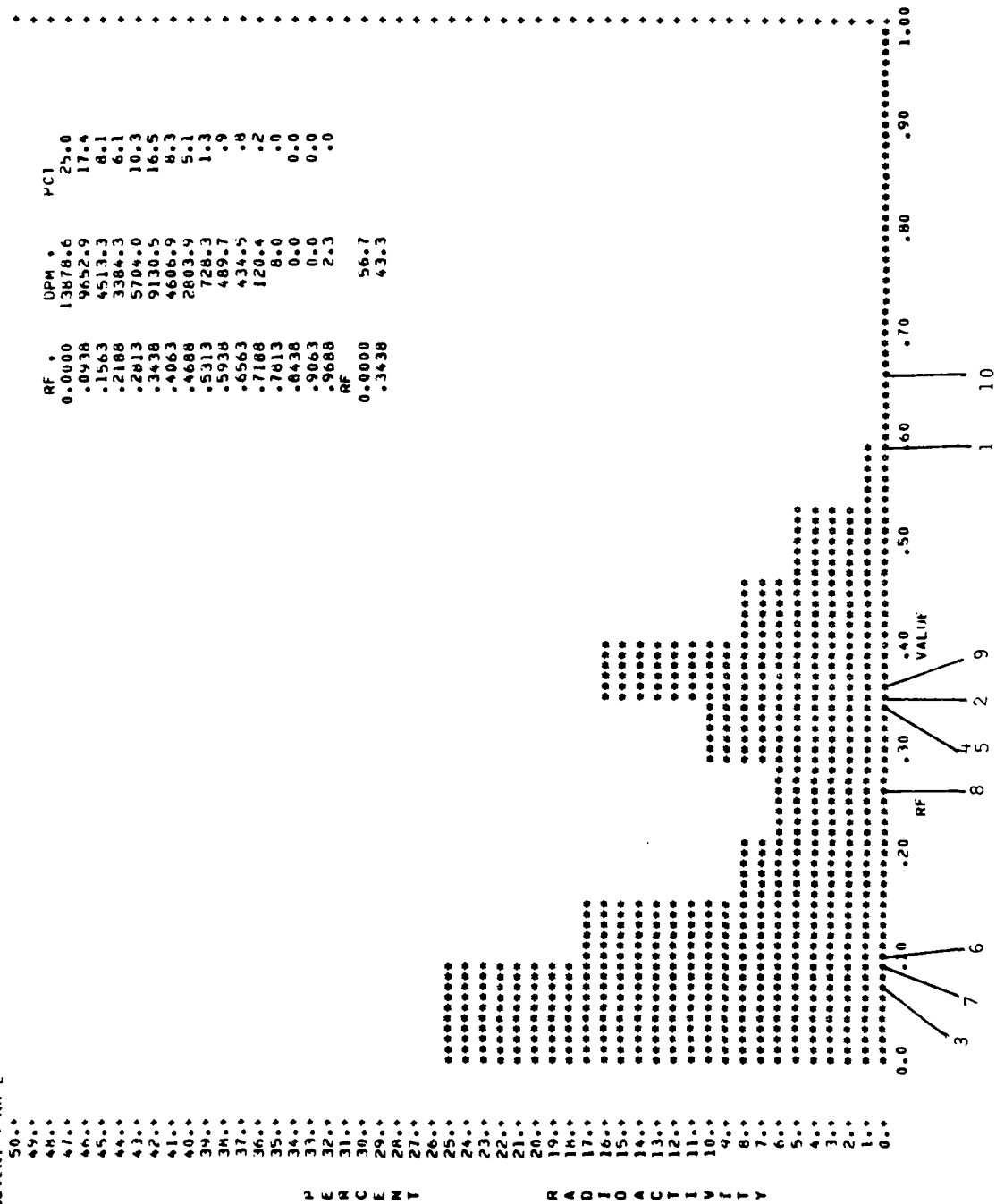


Figure 22-f-IX: Oral Treatment, Incubation with B-glucuronidase, Solvent IX.

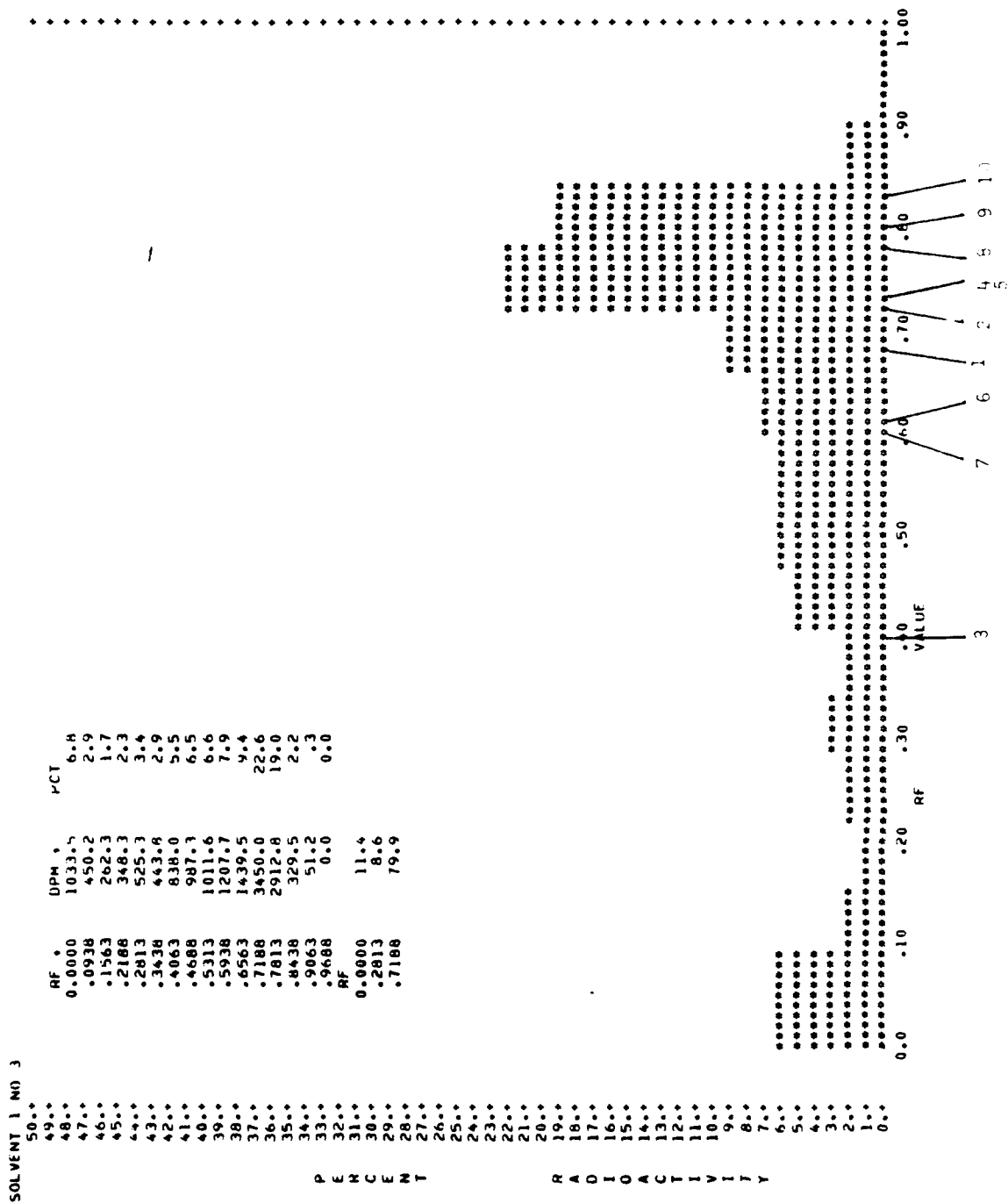


Figure 22-g-I: Dermal Application, Incubation with Water, Solvent 1.

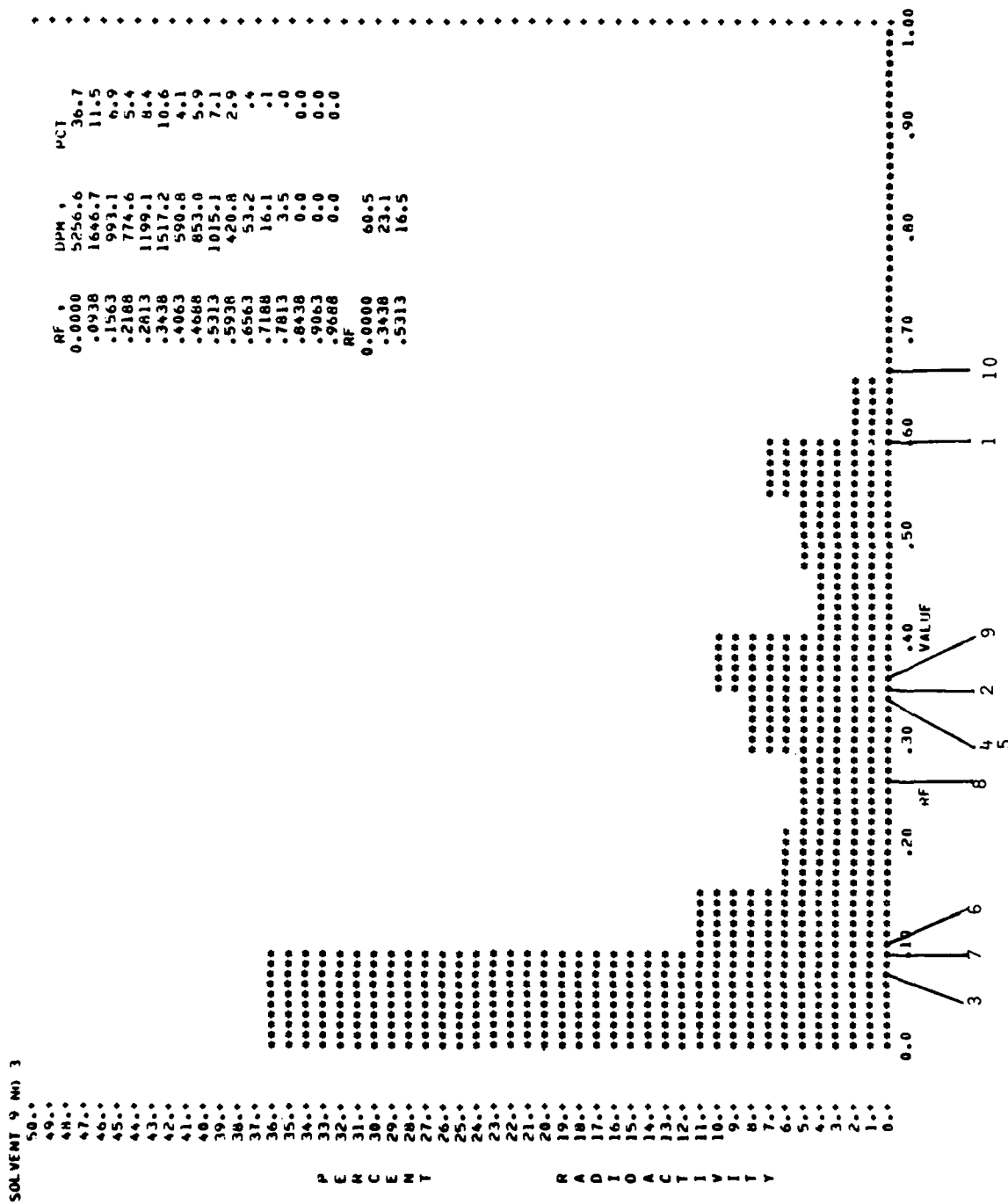


Figure 22-g-IX: Dermal Application, Incubation with Water, Solvent IX.

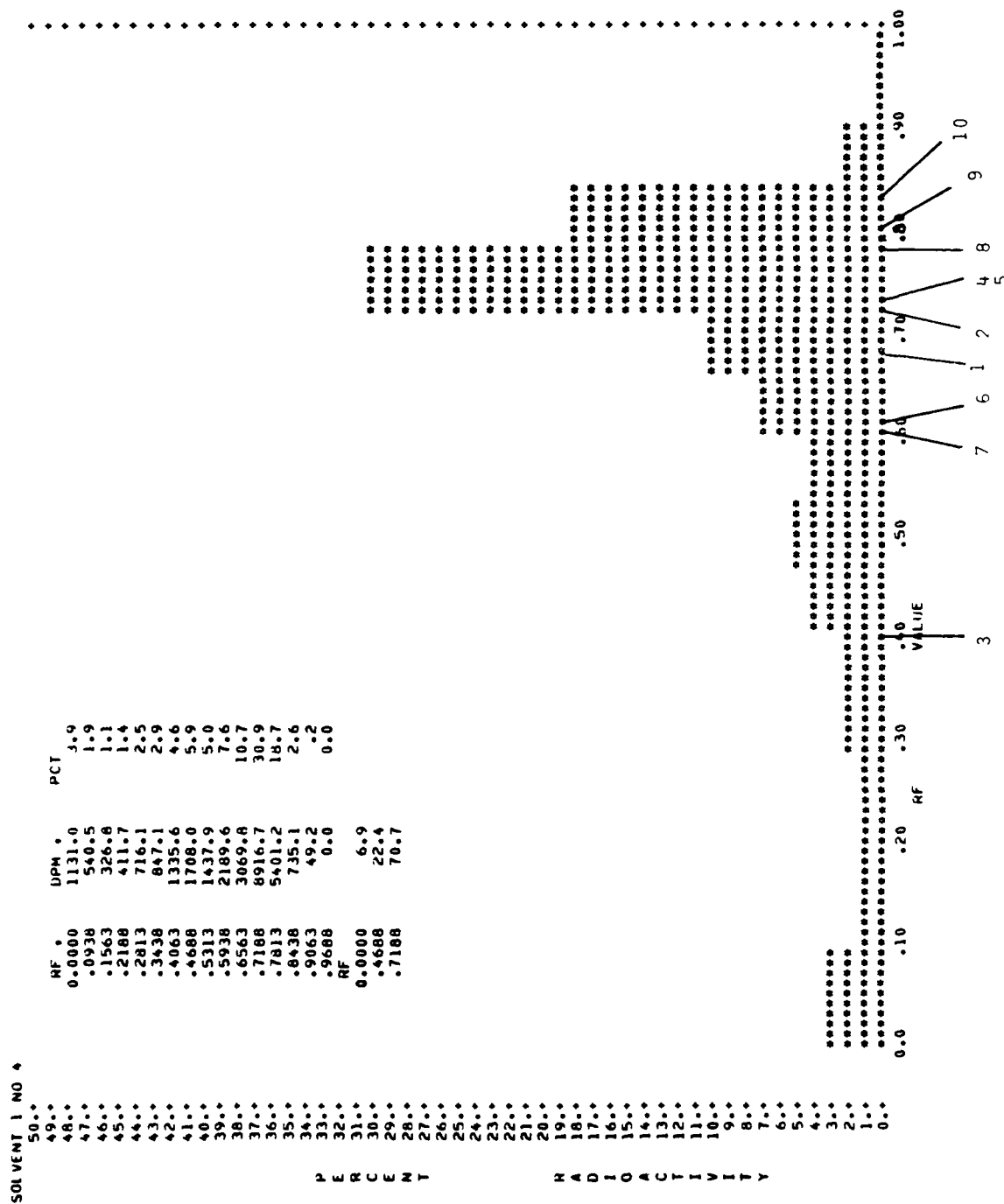


Figure 22-h-I: Dermal Application, Incubation with B-glucuronidase, Solvent I.

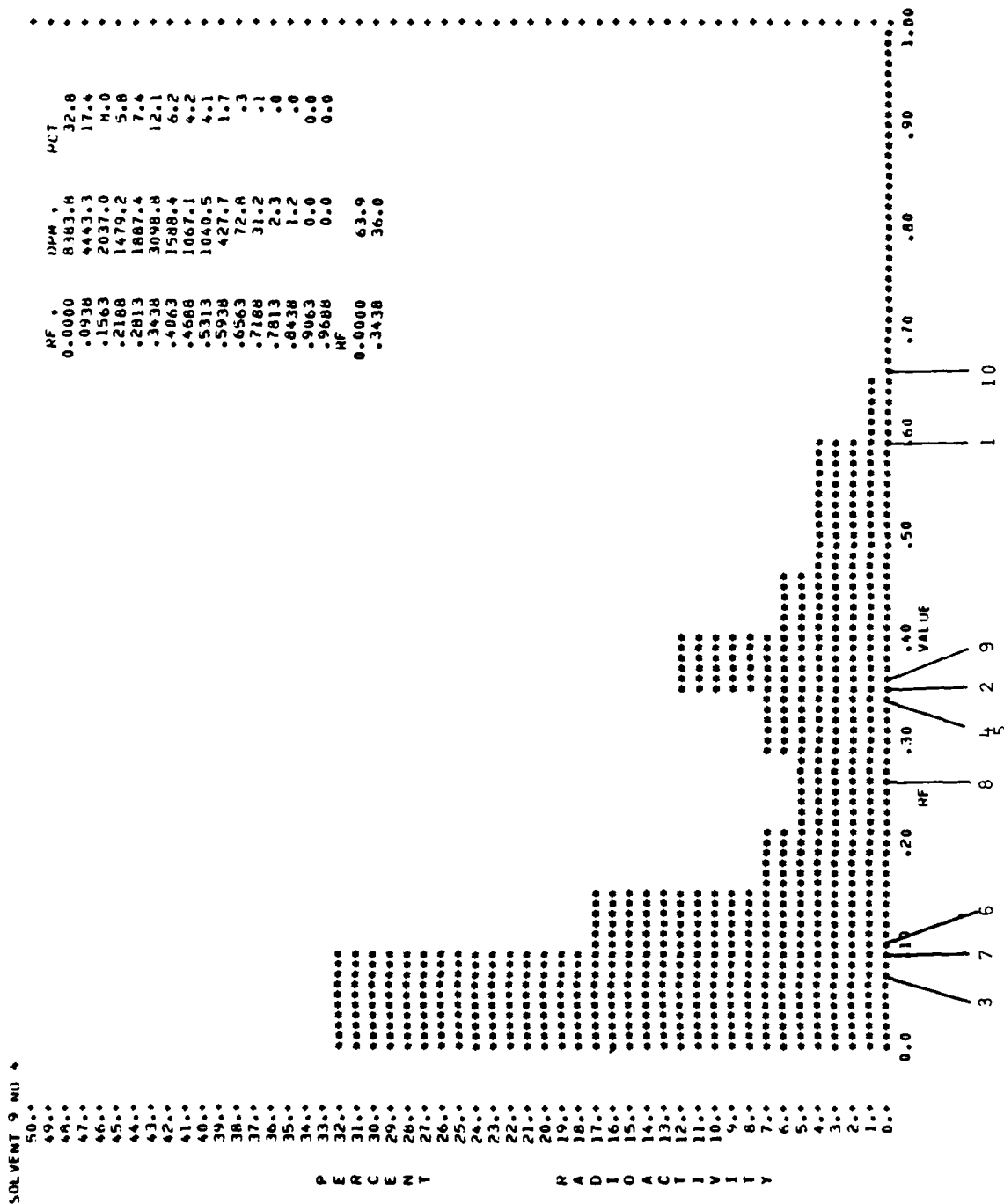


Figure 22-h-IX: Dermal Application, Incubation with B-glucuronidase, Solvent IX.

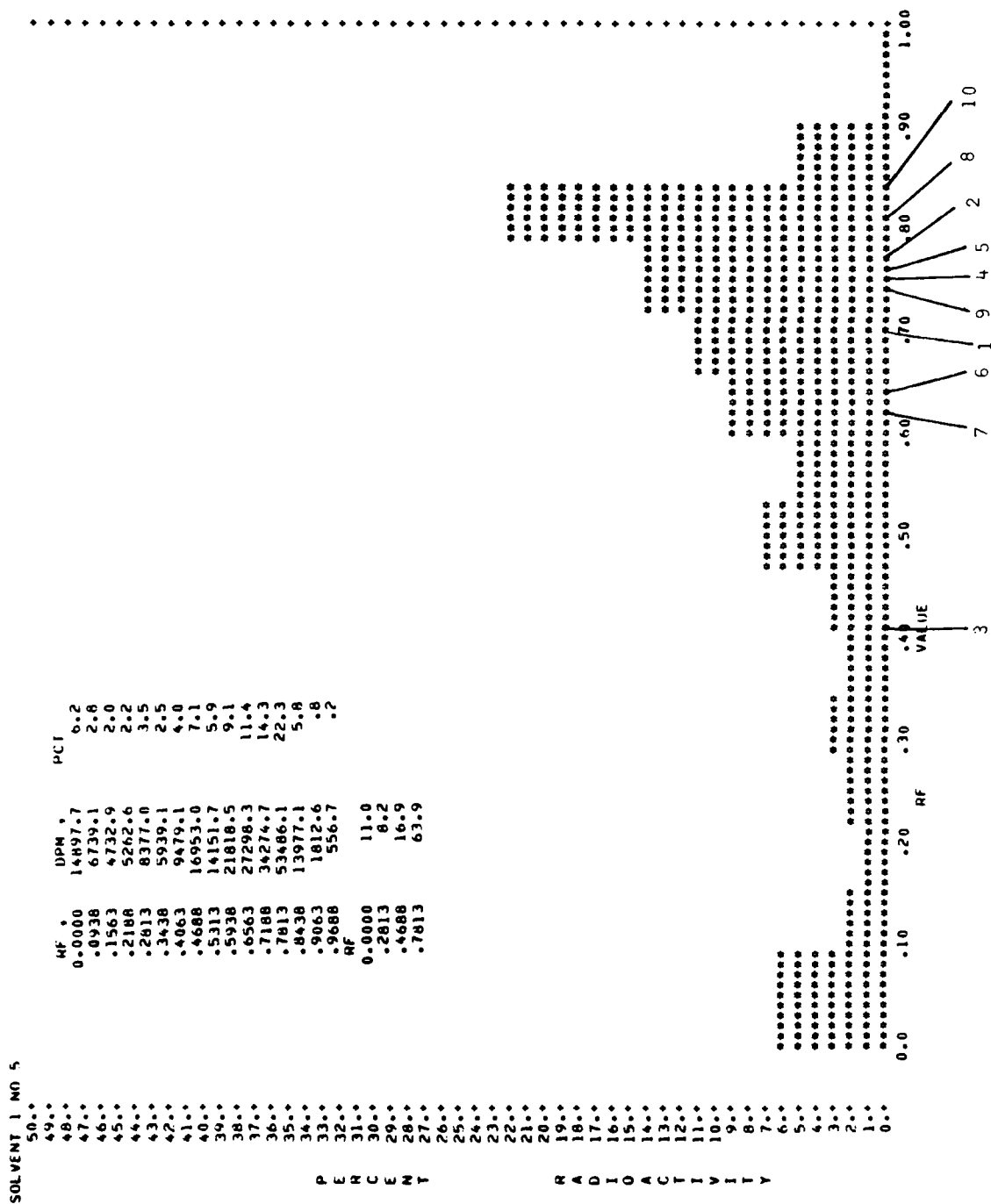


Figure 22-k-I: Oral Treatment, Incubation with Water, Solvent I.

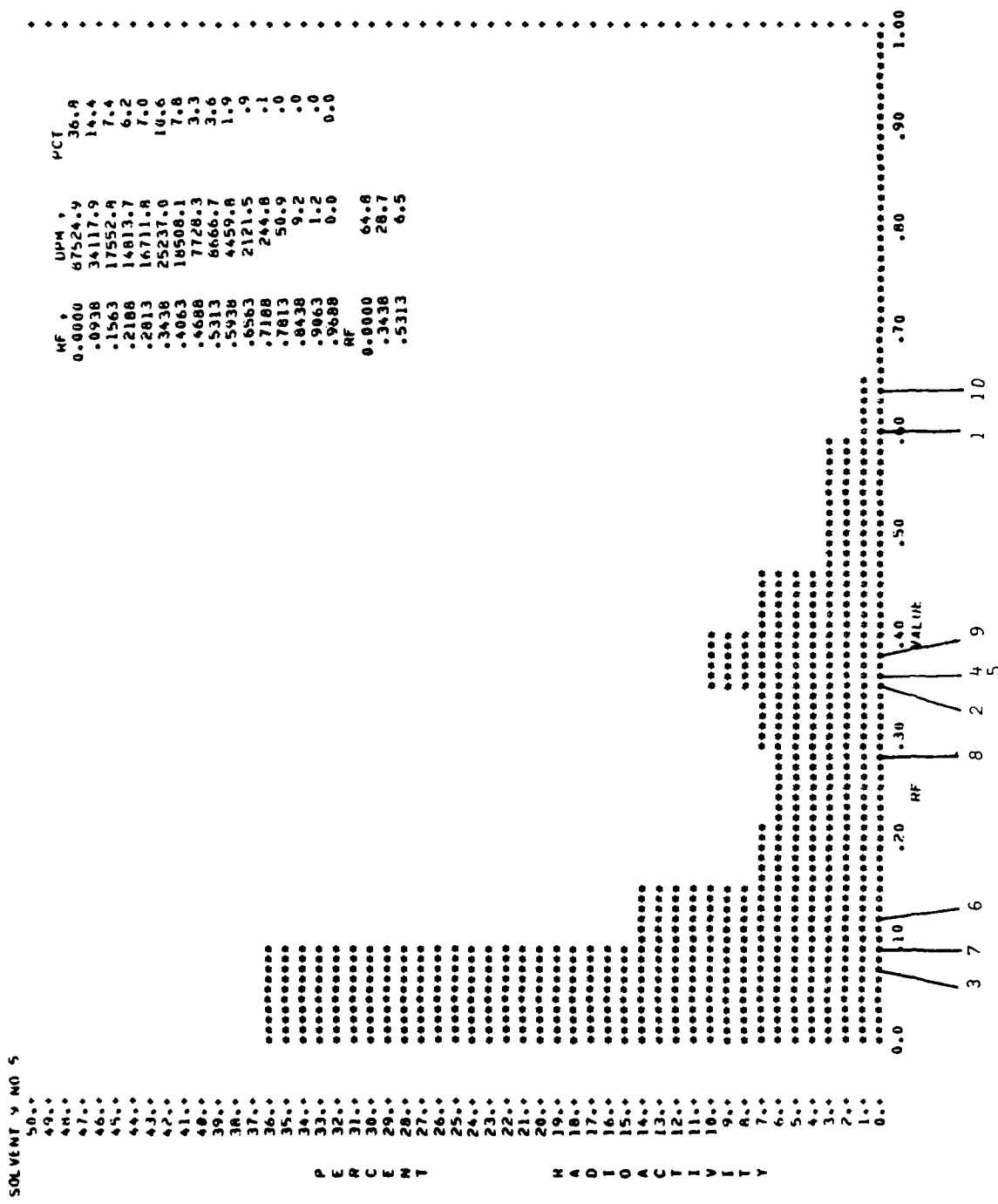


Figure 22-k-IX: Oral Treatment, Incubation with Water, Solvent IX.

SOLVENT 1 NO 7

50.0	MF	DPM	PCT
49.0	0.0000	3342.9	5.7
48.0	.0938	1249.7	2.1
47.0	.1563	1012.6	1.7
46.0	.2188	1105.3	1.9
45.0	.2813	1953.8	3.3
44.0	.3438	1965.5	3.4
43.0	.4063	2264.9	3.9
42.0	.4688	3371.6	5.8
41.0	.5313	3091.6	5.3
40.0	.5938	4275.1	7.3
39.0	.6563	5647.8	9.7
38.0	.7188	8785.0	15.0
37.0	.7813	16888.9	28.9
36.0	.8438	3133.9	5.4
35.0	.9063	321.8	.6
34.0	.9688	22.9	.0
33.0	RF		
32.0	0.0000	9.6	
31.0	.4688	23.5	
30.0	.7813	66.9	
29.0			
28.0			
27.0			
26.0			
25.0			
24.0			
23.0			
22.0			
21.0			
20.0			
19.0			
18.0			
17.0			
16.0			
15.0			
14.0			
13.0			
12.0			
11.0			
10.0			
9.0			
8.0			
7.0			
6.0			
5.0			
4.0			
3.0			
2.0			
1.0			
0.0			

P E R C E N T

M A D I O A C Y I V I Y

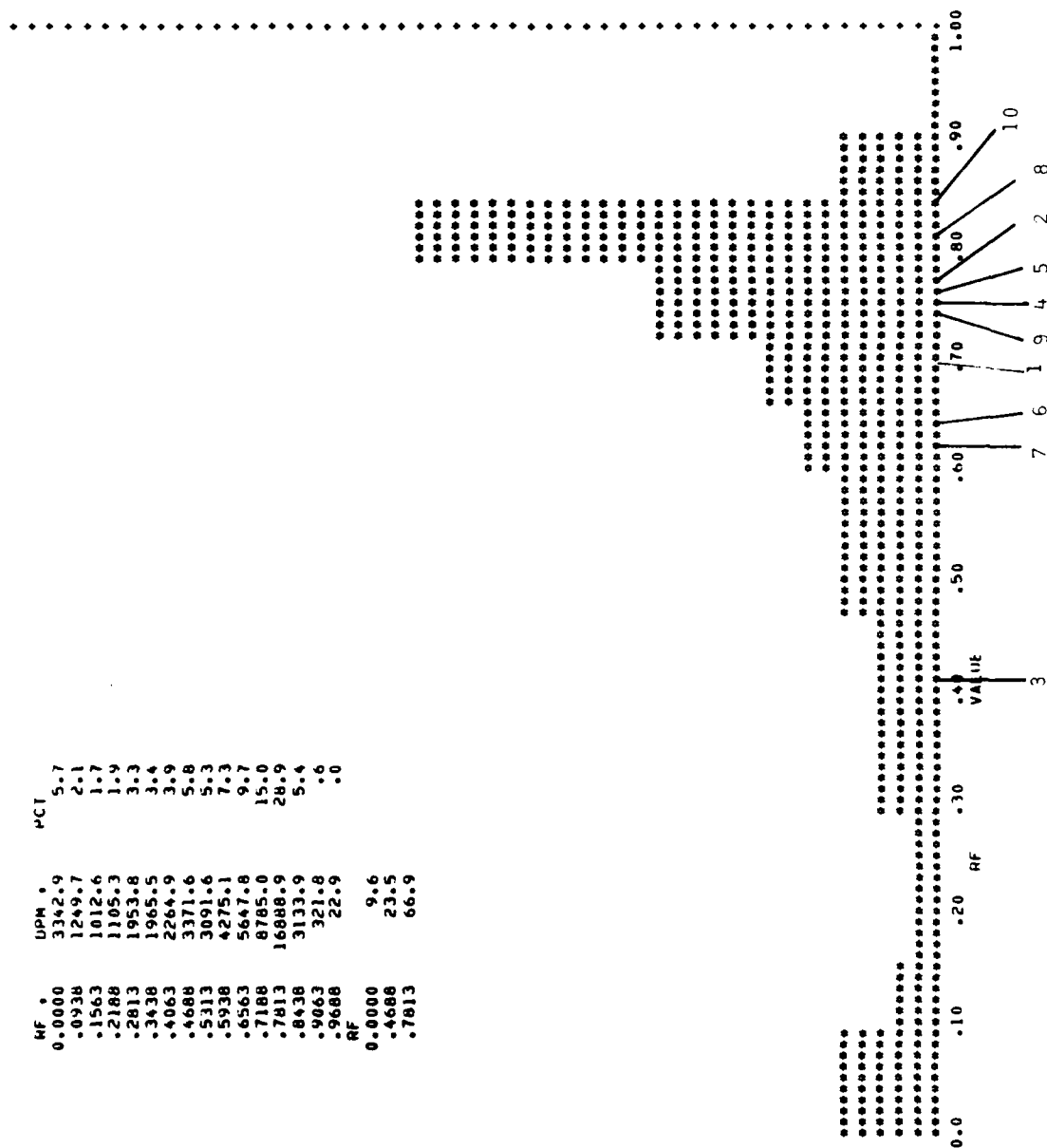


Figure 22-1-I: Dermal Application, Incubation with Water, Solvent I.

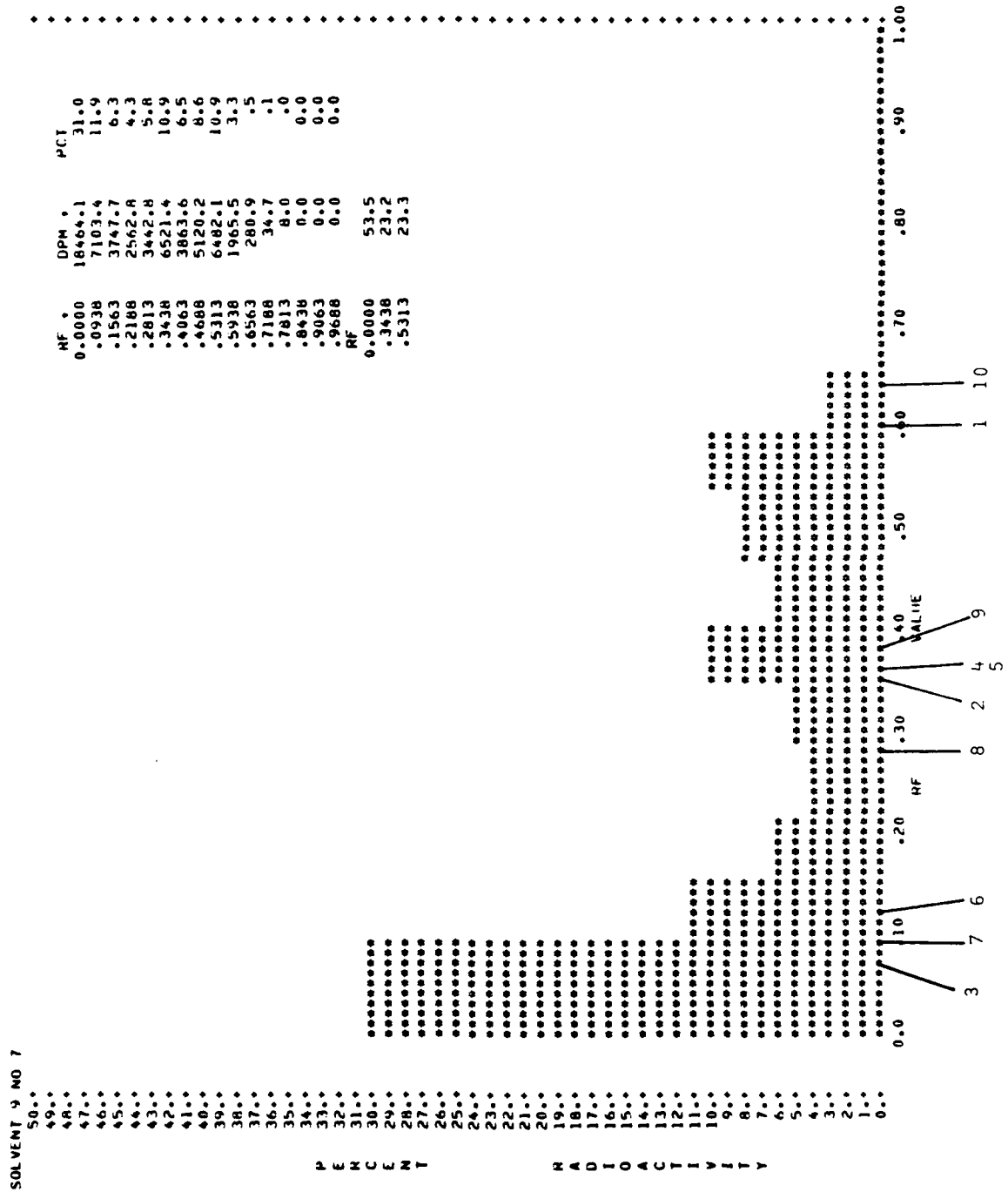


Figure 22-1-IX: Dermal Application, Incubation with Water, Solvent IX.

Figure 23: TLC of the Aqueous Non-Extractable Material Remaining After Extraction of TNT-Urine from Rats, Rabbits and Dogs with Ethyl Acetate. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrobenzene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 23 follows

SOLVENT 1 NO 15

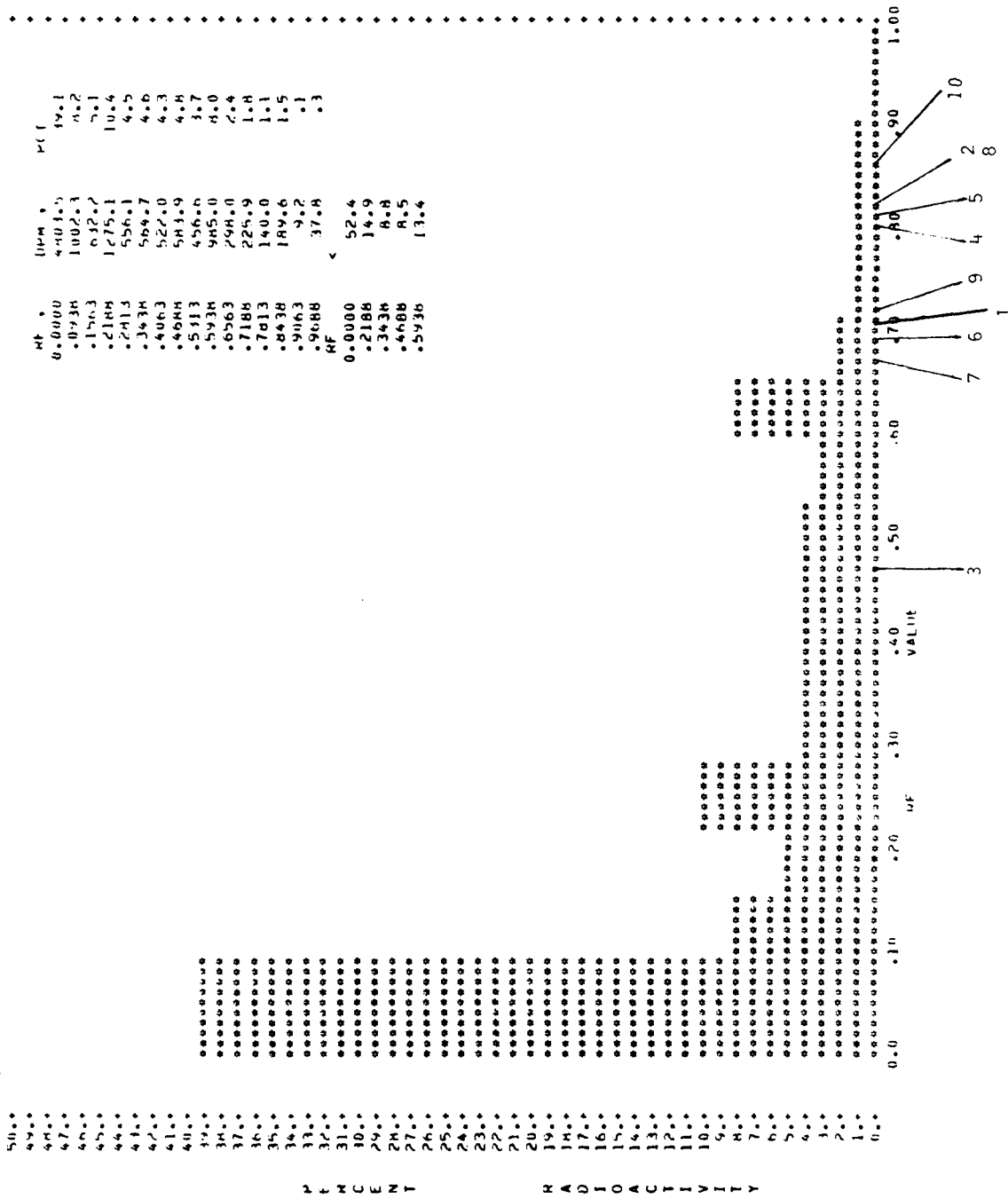
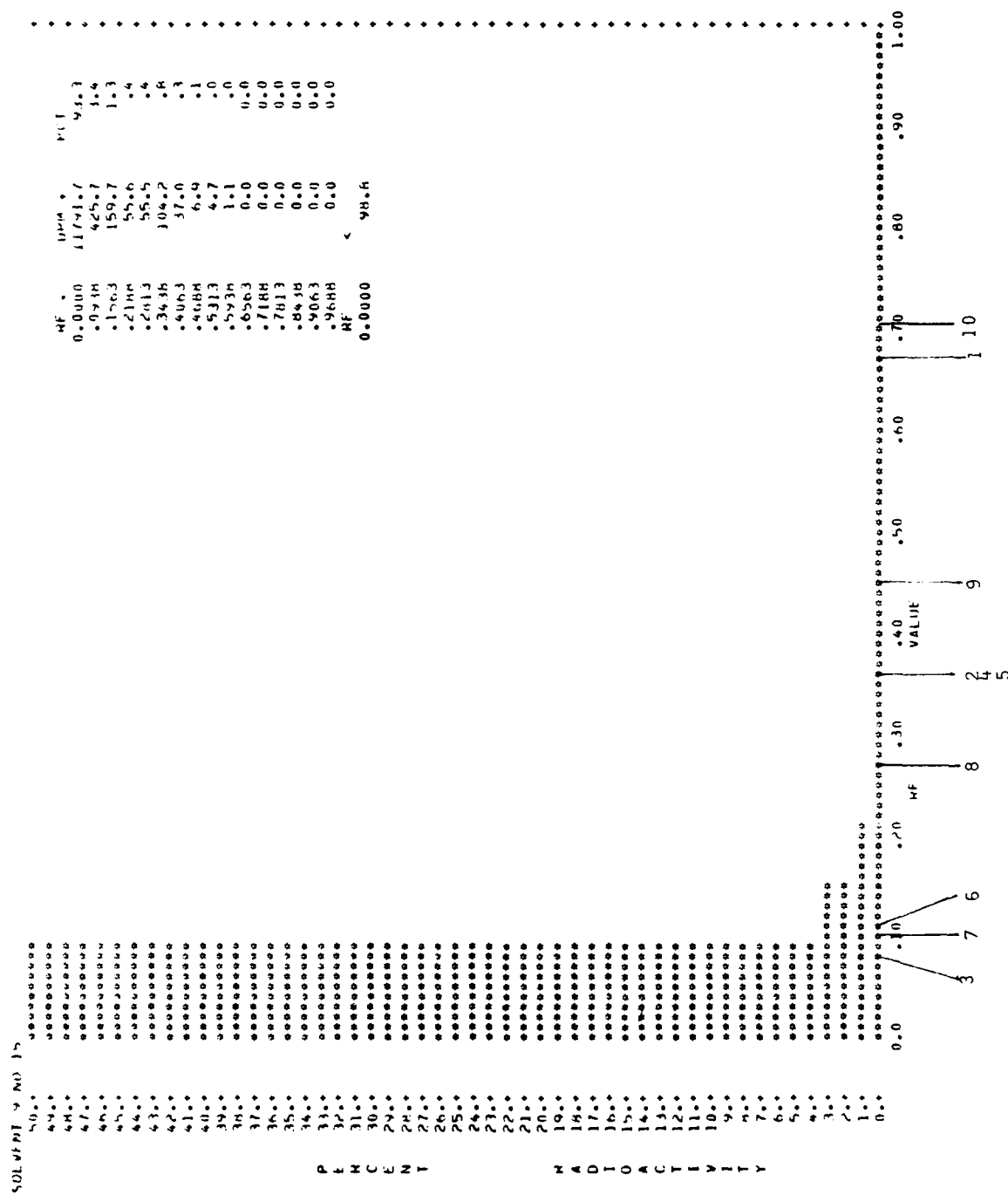


Figure 23-a-I: Male Rats, Oral Treatment, Solvent I



SOLVENT 1 NO 9

70.0	0.0000	1604.4	30.6
49.0	.0734	2501.2	5.4
48.0	.1563	2358.1	8.3
47.0	.2188	3058.4	10.4
46.0	.2413	1783.2	6.3
45.0	.3638	1463.3	7.1
44.0	.4063	1827.6	6.5
43.0	.4688	1359.5	4.4
42.0	.5313	2286.2	4.1
41.0	.5938	1104.4	3.4
40.0	.6563	730.3	2.6
39.0	.7188	641.7	2.4
38.0	.7813	480.2	1.7
37.0	.8438	12.6	.0
36.0	.9063	1.2	.0
35.0	.9688	0.0	0.0
34.0	MF	<	
33.0	0.0000	47.8	
32.0	.2188	22.2	
31.0	.4063	11.3	
30.0	.5313	18.7	

P L W C E N Y

A U I O A C T I V I T Y

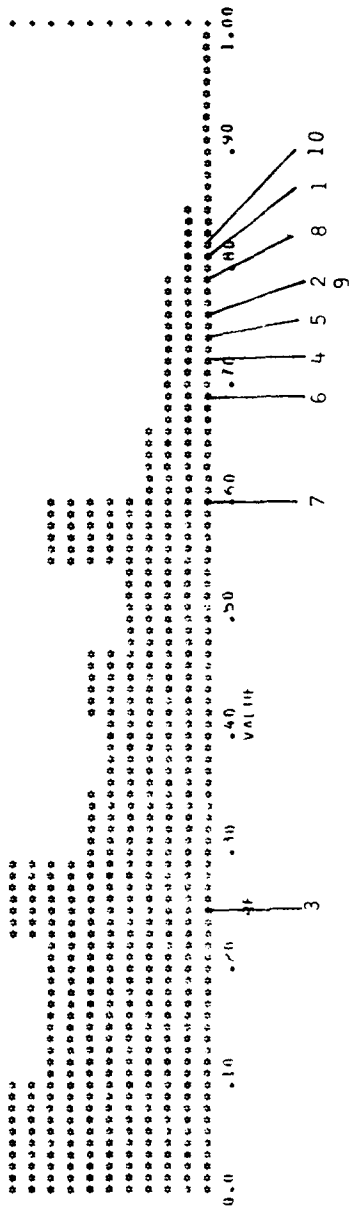


Figure 23-b-I: Female Rats, Oral Treatment, Solvent I

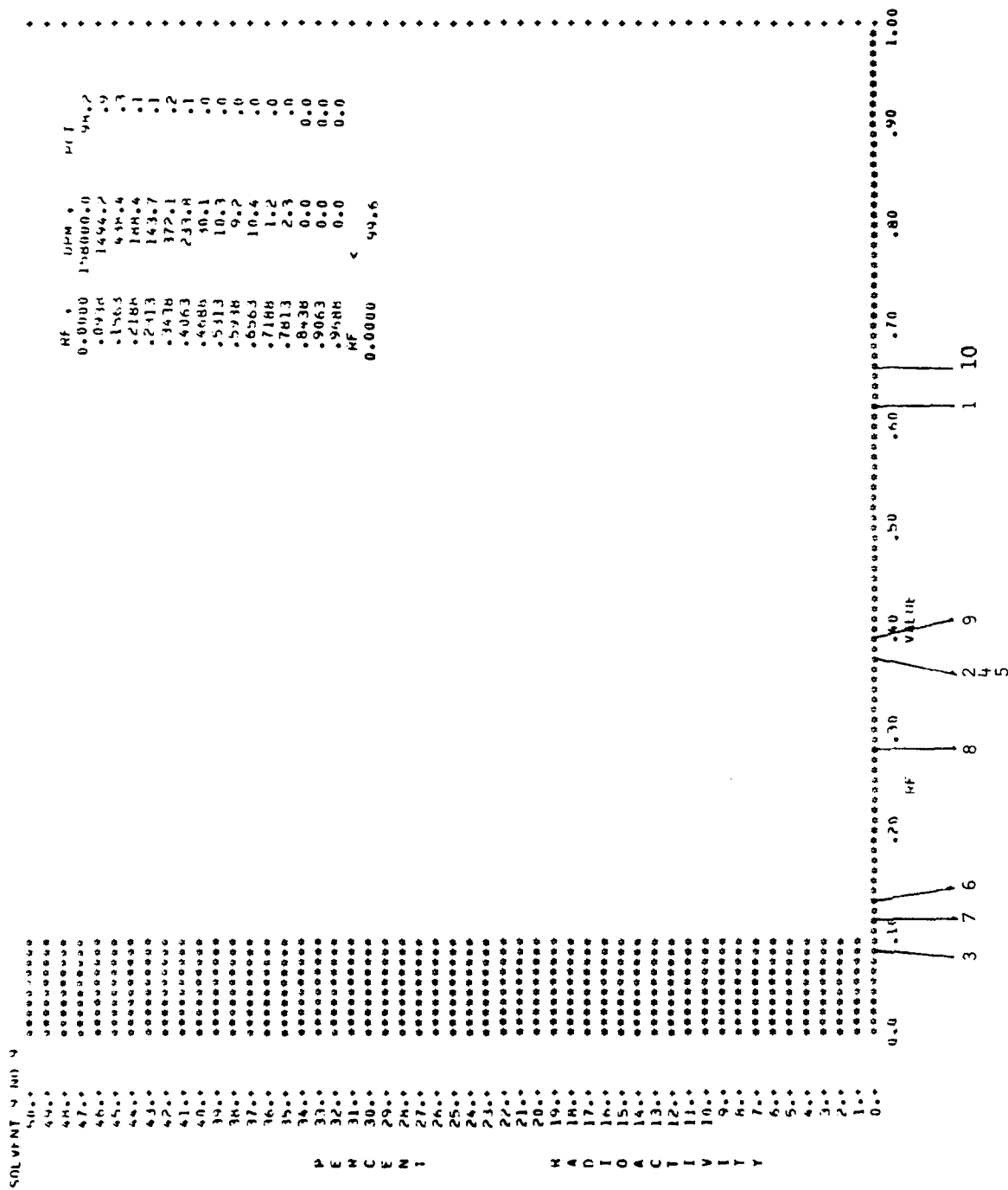


Figure 23-b-IX: Female Rats, Oral Treatment, Solvent IX

	MT	1974	1977
0.0000	0.0000	842.14.6	42.1
0.93H	0.93H	2430.0	12.1
1.563	1.563	1227.7	6.1
2.185	2.185	880.7	4.4
2.813	2.813	949.1	4.7
3.43H	3.43H	1094.9	5.5
4.063	4.063	1345.3	6.7
4.68H	4.68H	1081.4	5.6
5.313	5.313	1443.3	7.2
5.93H	5.93H	475.6	2.4
6.563	6.563	332.2	1.7
7.18H	7.18H	181.5	.9
7.813	7.813	132.9	.7
8.43H	8.43H	6.9	.0
9.063	9.063	0.0	0.0
9.68H	9.68H	0.0	0.0
RF	RF	<	
0.0000	0.0000	64.4	
4.063	4.063	22.3	
5.313	5.313	12.4	

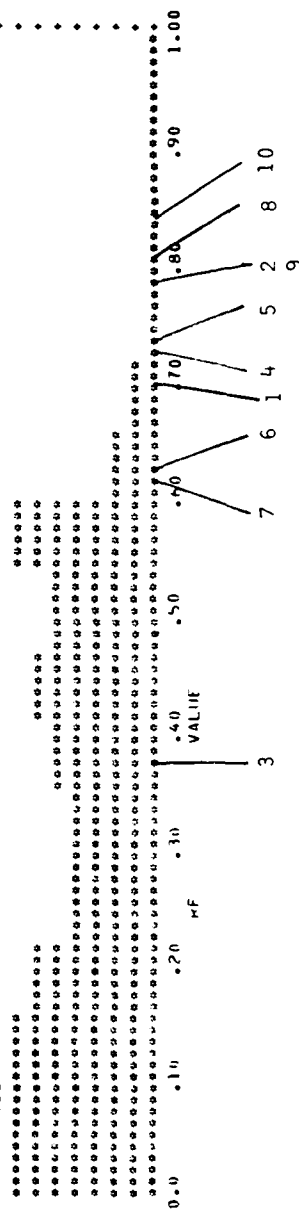


Figure 23-c-I: Male Rats, Dermal Application, Solvent I

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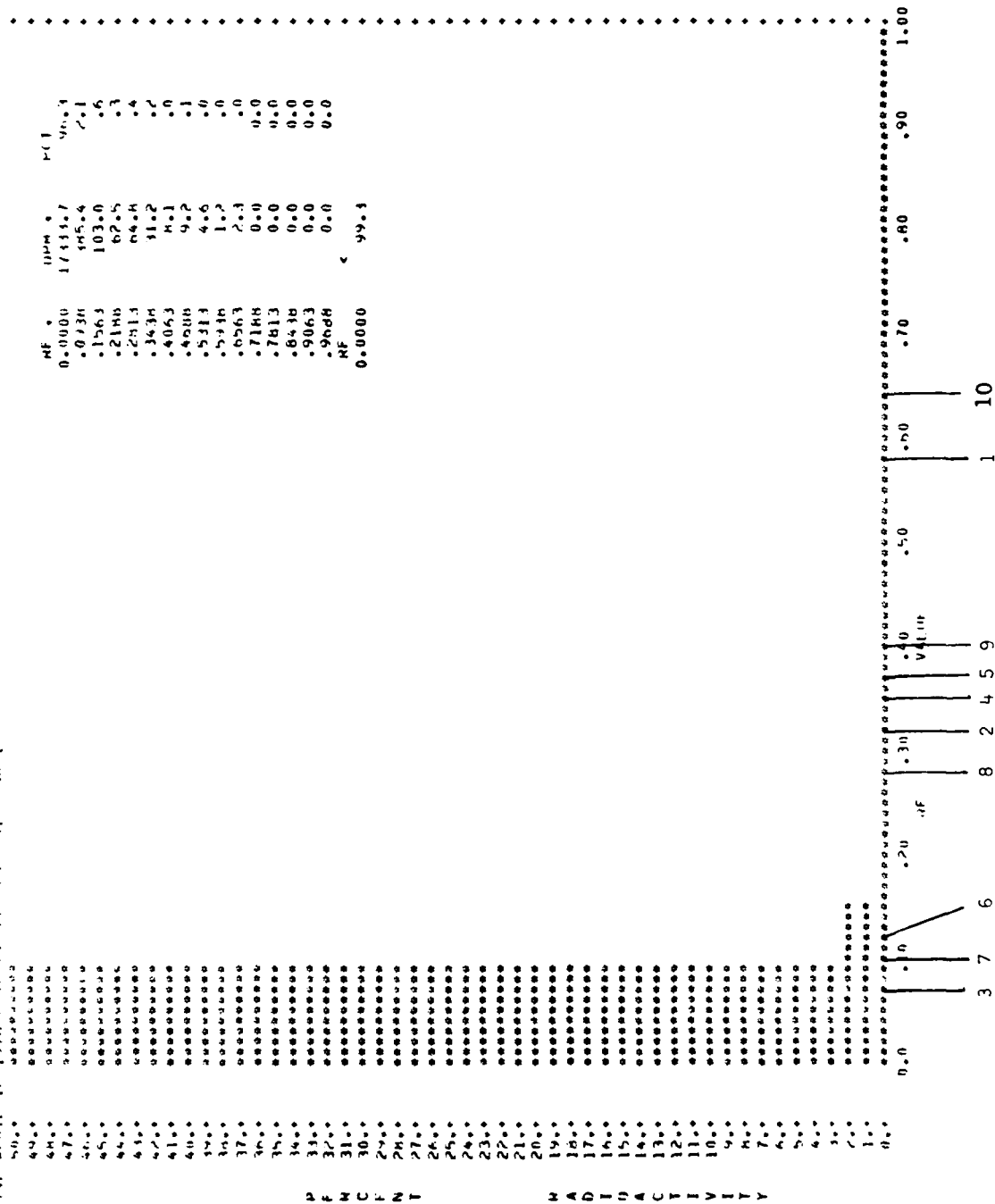


Figure 23-c-IX: Male Rats, Dermal Application, Solvent I

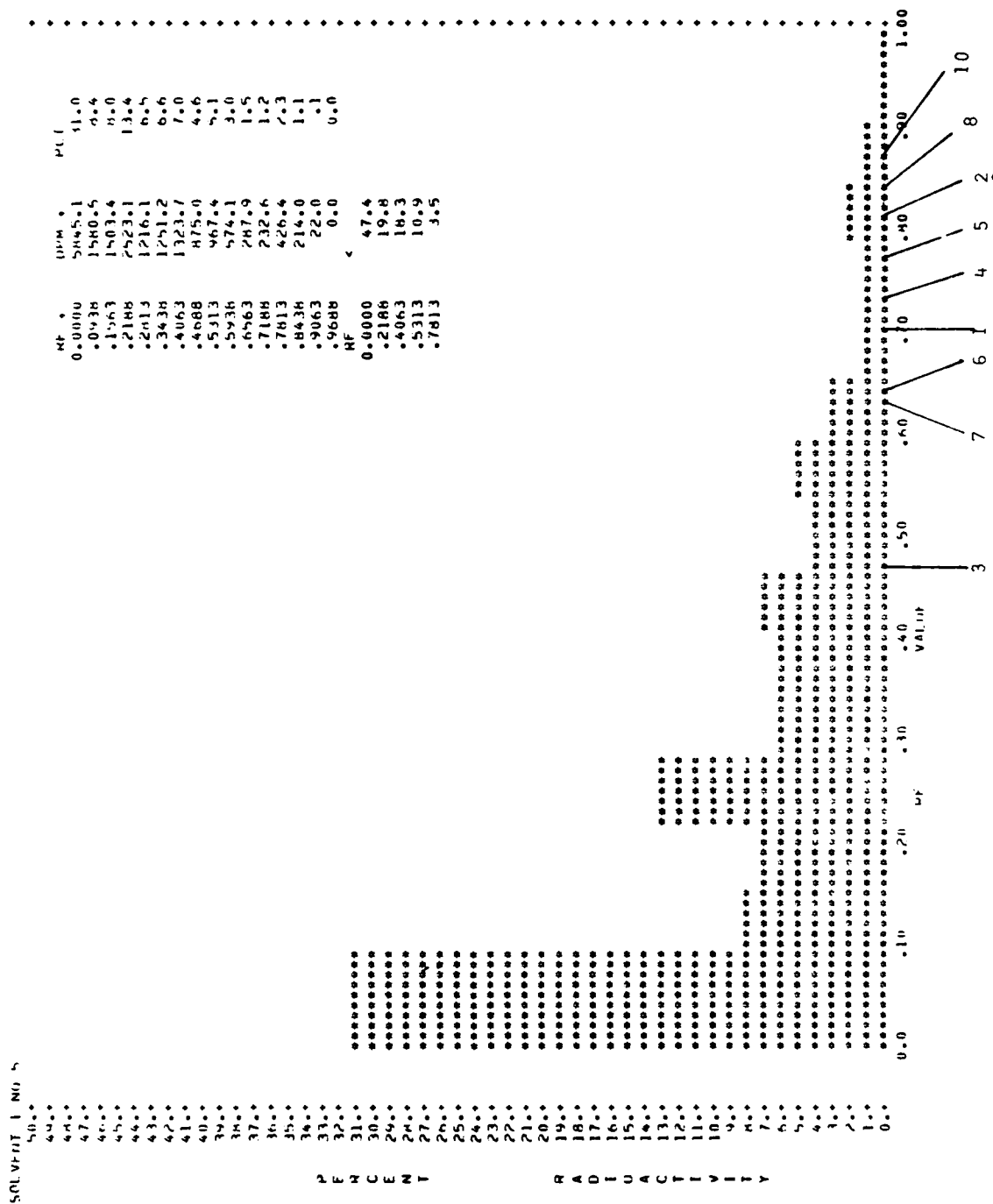


Figure 23-d-I: Female Rats, Dermal Application, Solvent I

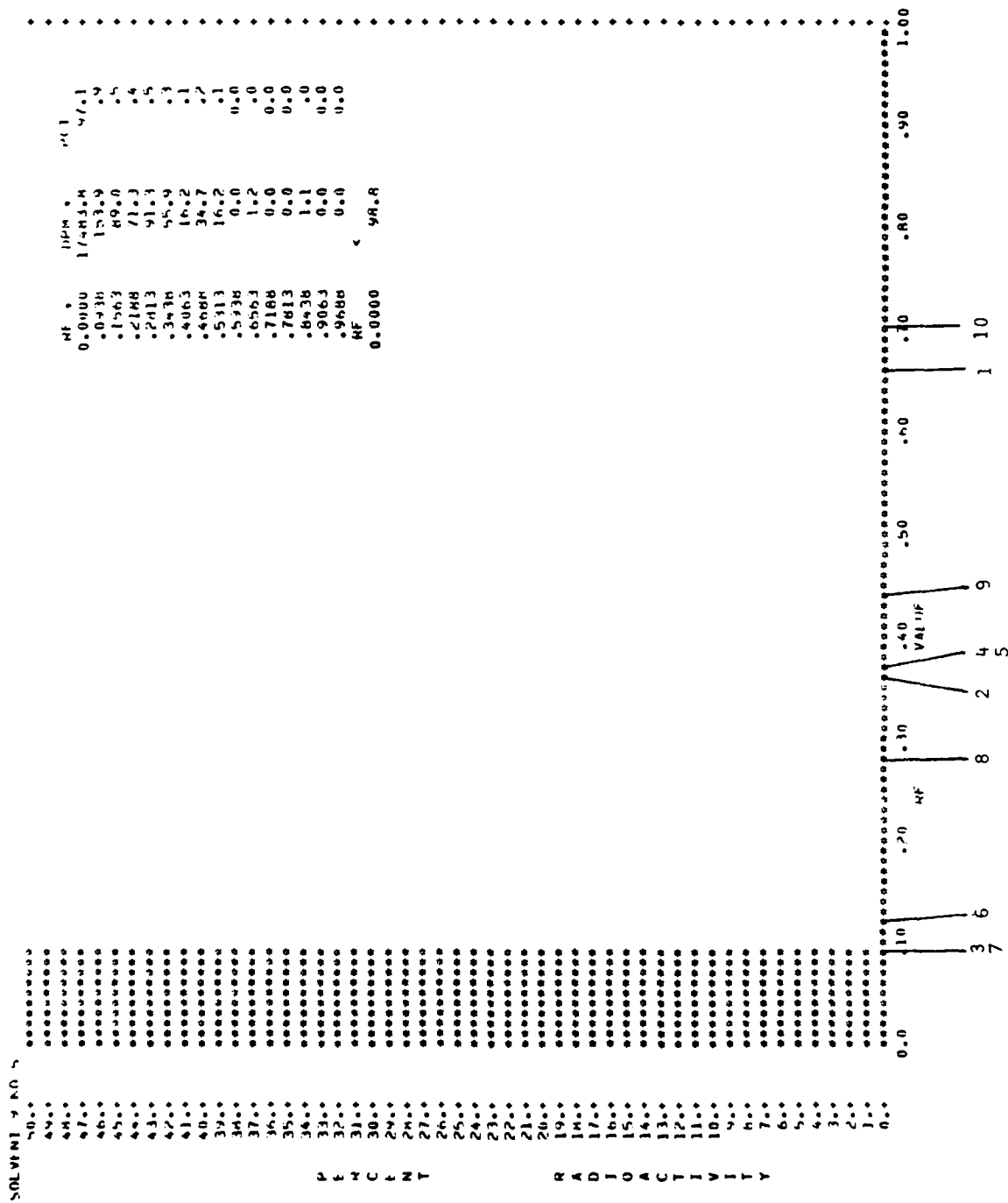


Figure 23-d-IX: Female Rats, Dermal Application, Solvent IX

SOLVENT I PNO 14

P	50.0	0.0000	10.0	30.0
E	48.0	.0438	3965.3	12.5
H	47.0	.1563	1998.8	6.3
C	46.0	.2188	1734.1	5.5
N	45.0	.2413	1576.1	6.2
Y	44.0	.3436	1403.4	4.4
	43.0	.4063	1216.4	4.8
	42.0	.4688	1080.4	4.7
	41.0	.5313	1225.7	3.9
	40.0	.5938	3904.0	12.3
	39.0	.6564	898.9	4.4
	38.0	.7188	530.1	1.7
	37.0	.7813	892.8	2.8
	36.0	.8438	618.1	2.0
	35.0	.9063	15.0	.0
	34.0	.9688	2.3	.0
	33.0	0.0000	54.3	
	32.0	.2813	10.6	
	31.0	.4063	13.4	
	30.0	.5938	16.9	
	29.0	.7813	4.8	

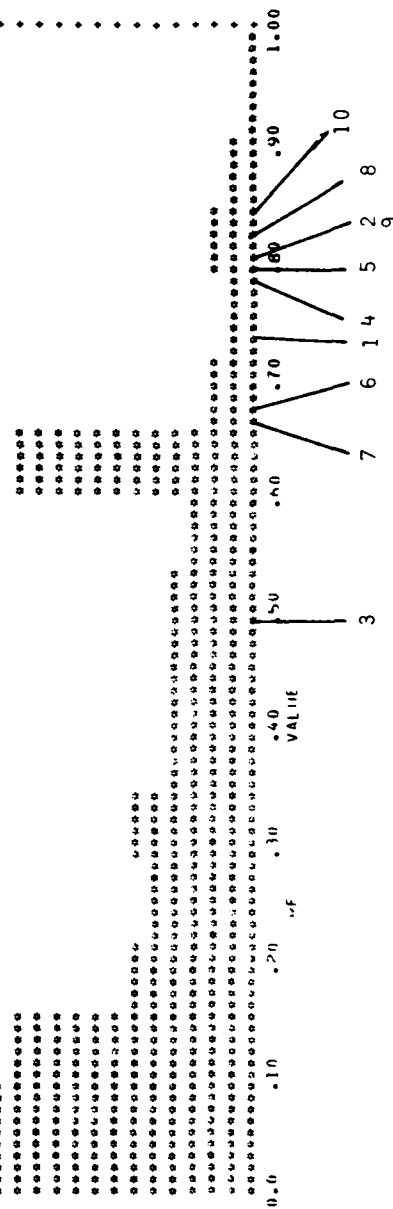


Figure 23-e-I: Male Rabbits, Oral Treatment, Solvent I

SOLVENT 9 NO 13

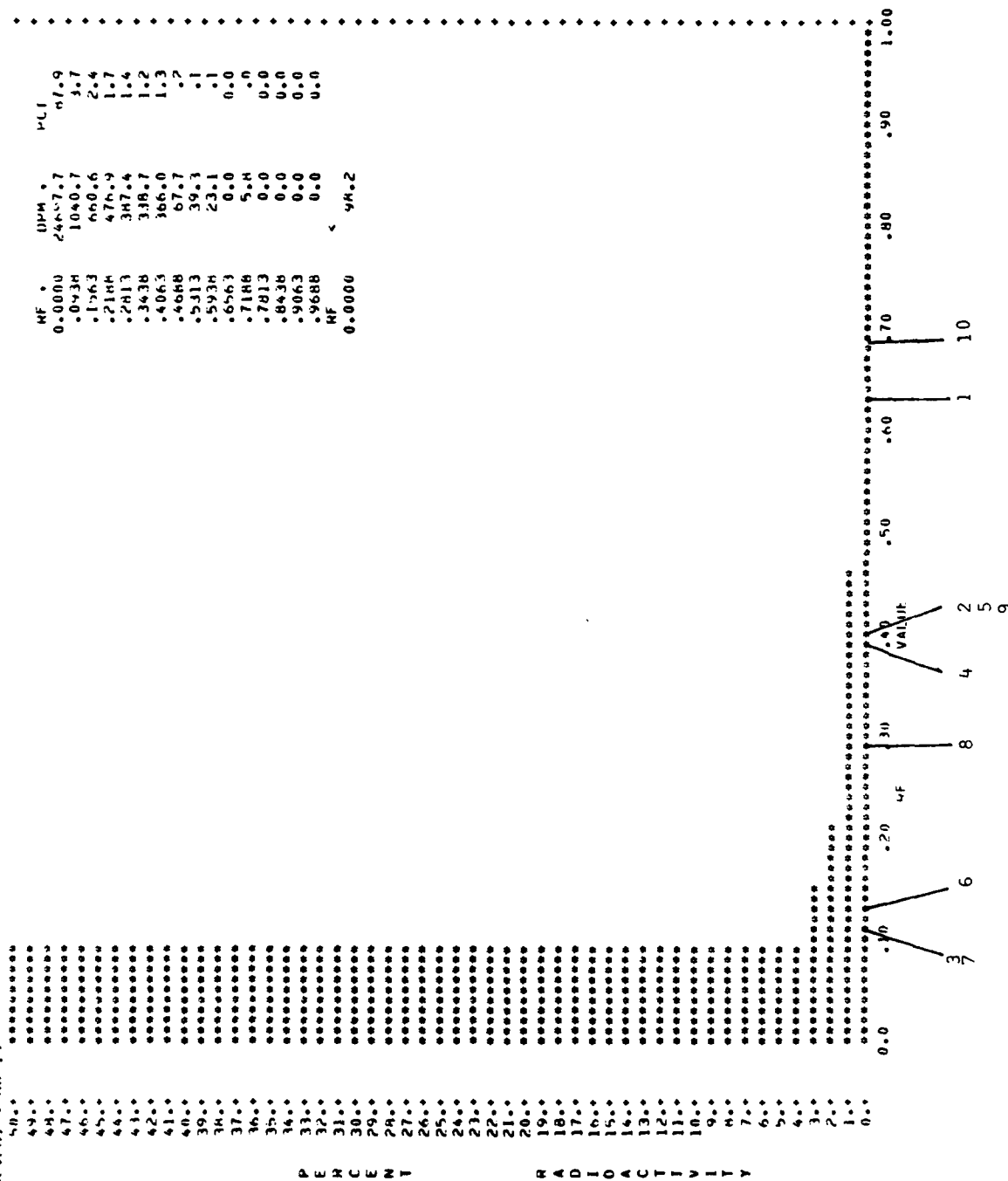


Figure 23-e-IX: Male Rabbits, Oral Treatment, Solvent IX

SOLVENT I NO 15

50.0
49.0
48.0
47.0
46.0
45.0
44.0
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

M E M C E M T

M A U I O A C T I V I T Y

MF .
0.0000
.0436
.1563
.2186
.2413
.3434
.4063
.4688
.5413
.5934
.6563
.7188
.7813
.8438
.9063
.9688
MF
0.0000
.2813
.4688
.5934
.7813

UPH .
14549.4
11471.1
4054.3
3414.5
5442.5
3406.4
3119.4
4367.6
4064.7
4775.8
1363.4
1189.5
2070.5
594.2
3.5
1.2
47.1
15.4
19.6
14.7
3.2

PCT
21.3
14.3
5.5
4.1
7.0
4.5
4.0
10.0
9.6
11.6
1.6
1.4
2.5
.7
.0
.0

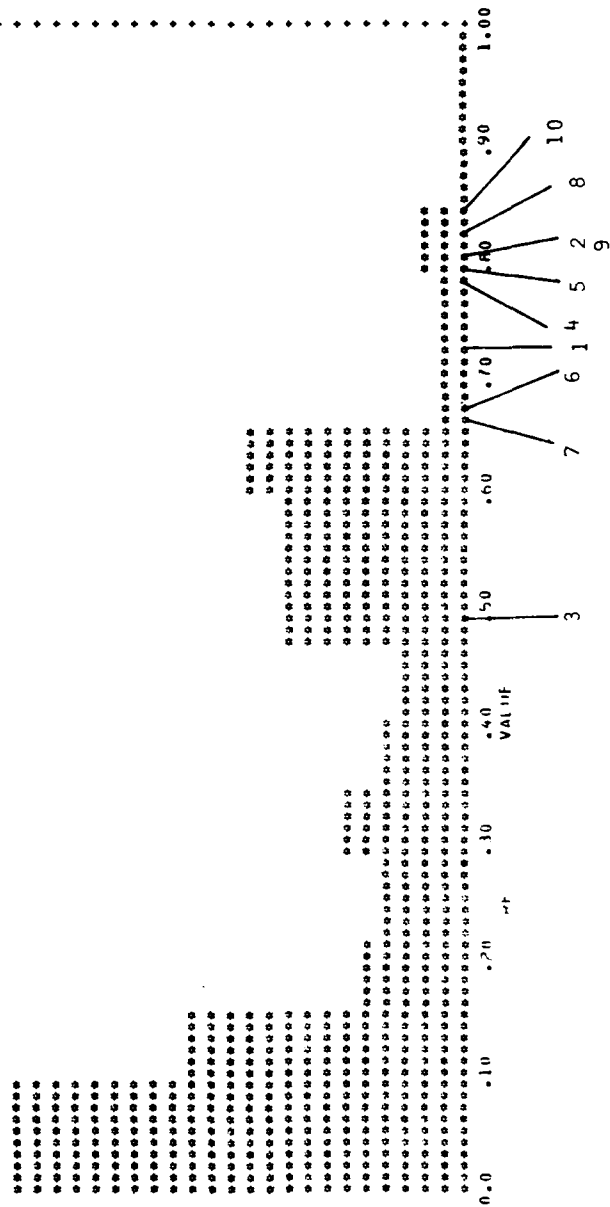


Figure 23-f-I: Male Rabbits, Dermal Application, Solvent I

SOLVENT 9 NO 15

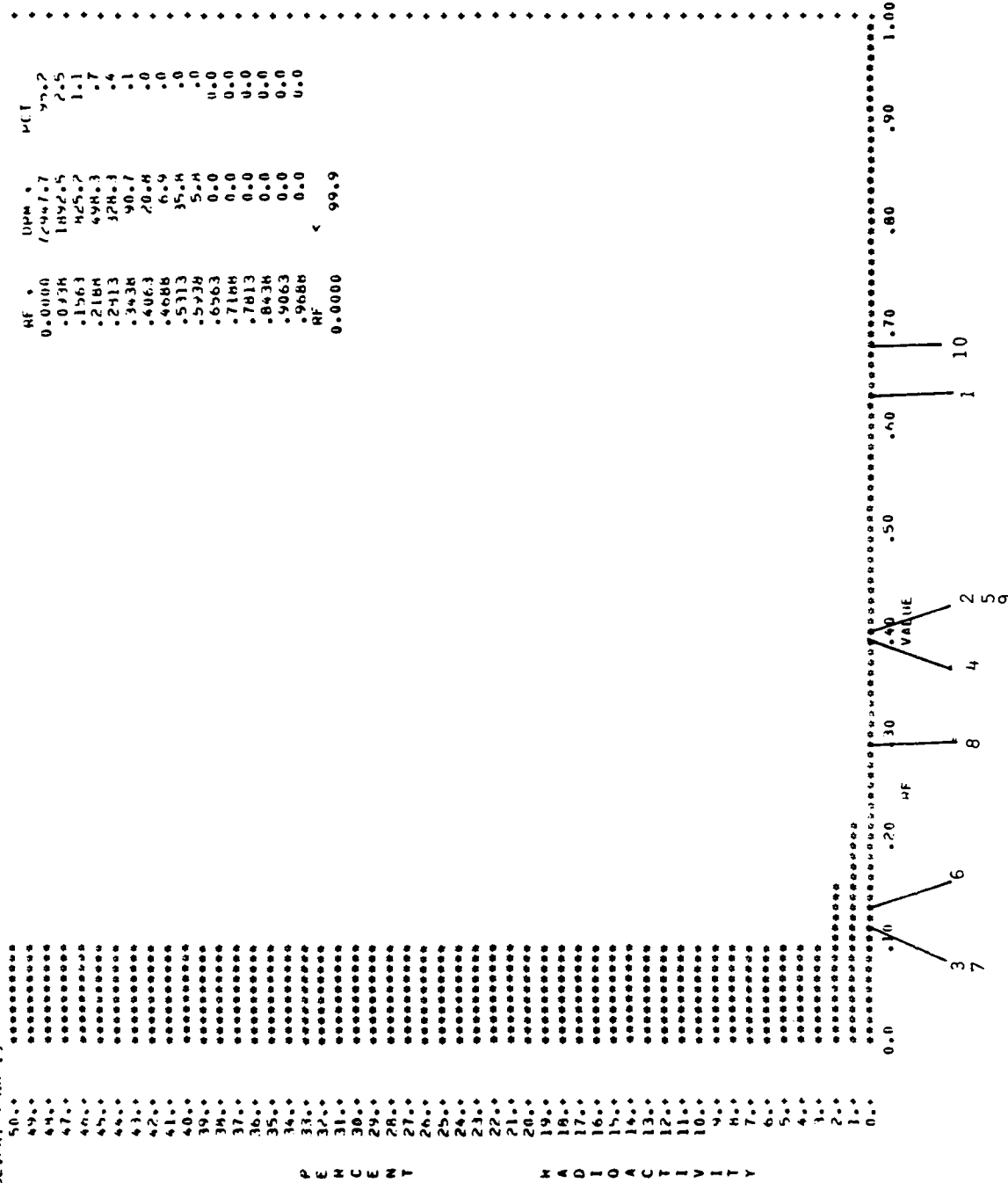


Figure 23-f-IX: Male Rabbits, Dermal Application, Solvent I

SOLVENT I NO. 1

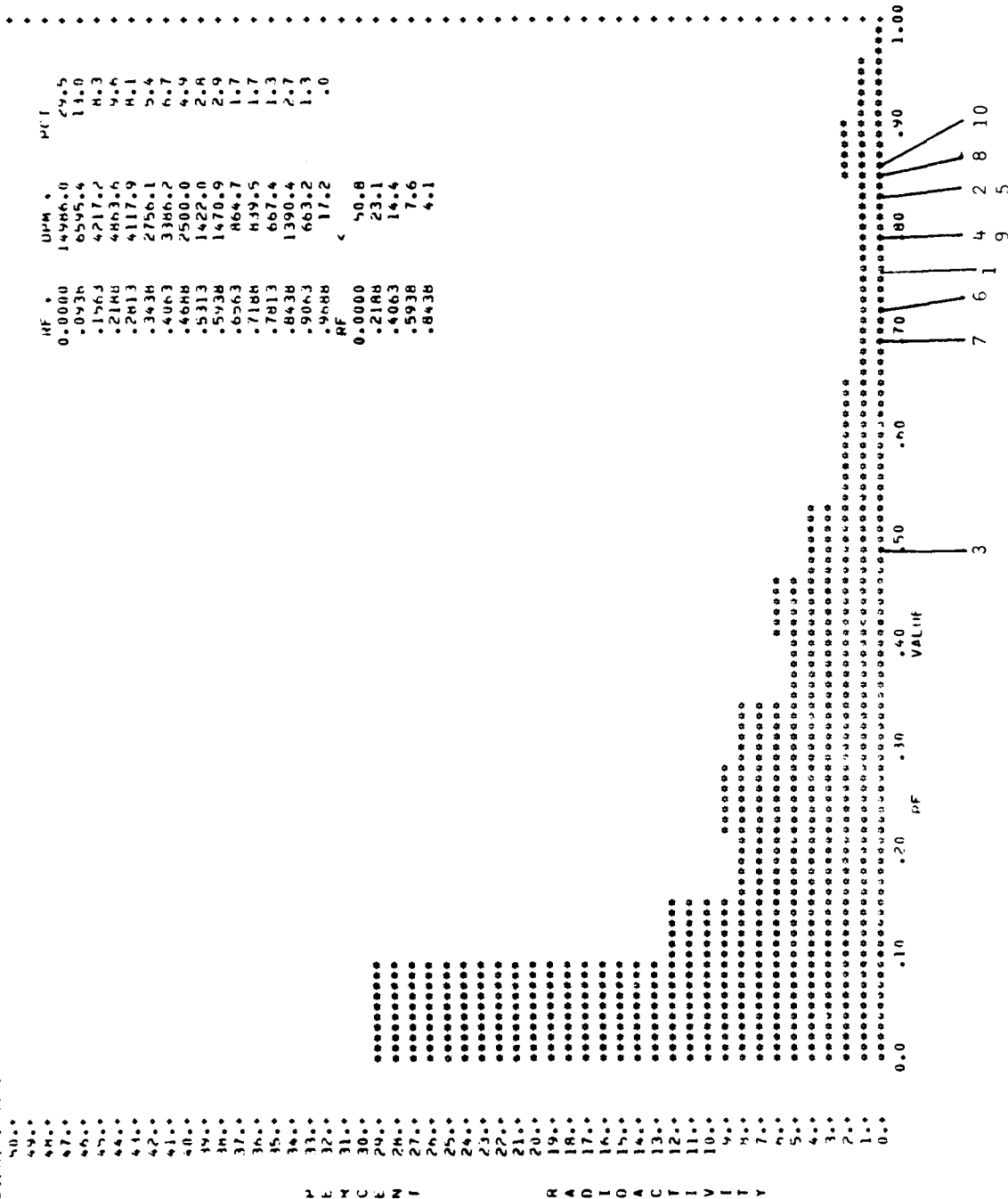
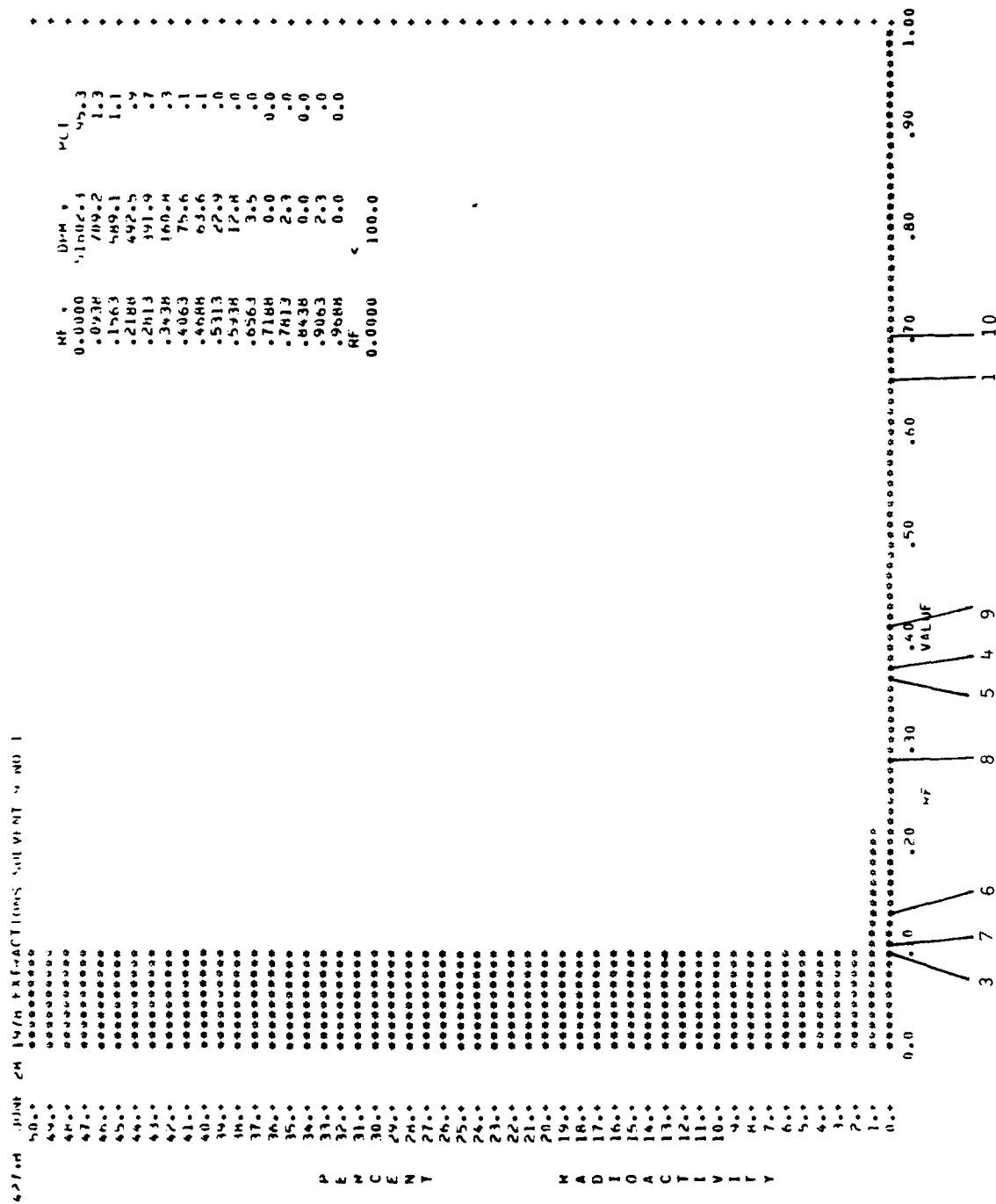


Figure 23-g-I: Male Dogs, Oral Treatment, Solvent I



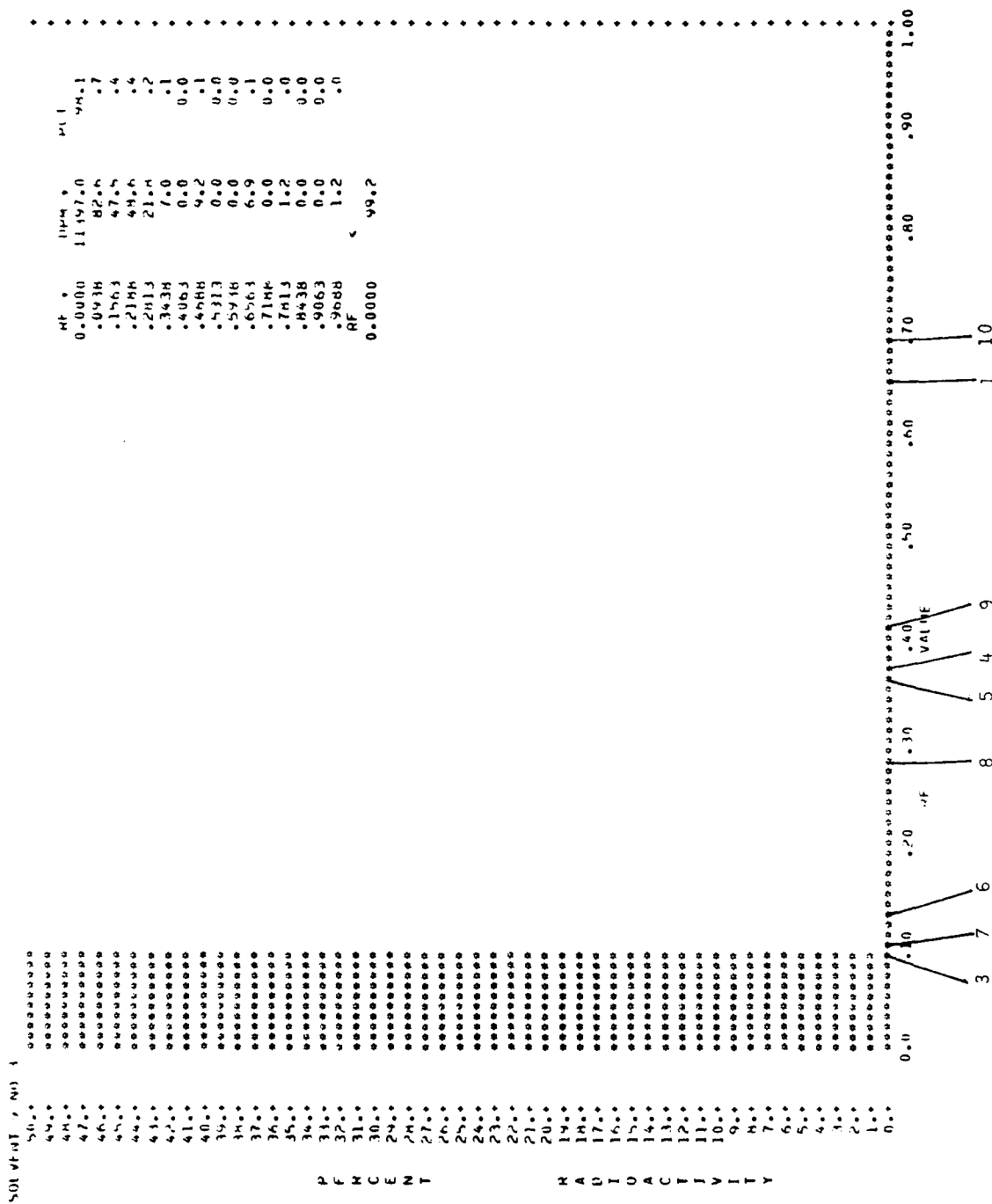


Figure 23-h-IX: Male Dogs, Dermal Application, Solvent IX

Figure 24: TLC of the Ethyl Acetate Extractable and Non-Extractable Material Obtained from Bile of Rabbits and Dogs Treated Orally or Dermally with ^{14}C -TNT. Reference standards are:

- | | |
|-------------------------------|---|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzylalcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-Dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-Dinitrotoluene | 10. 2,6,2,6'-Tetranitro-4,4'-azoxytoluene |

Figure 24 follows

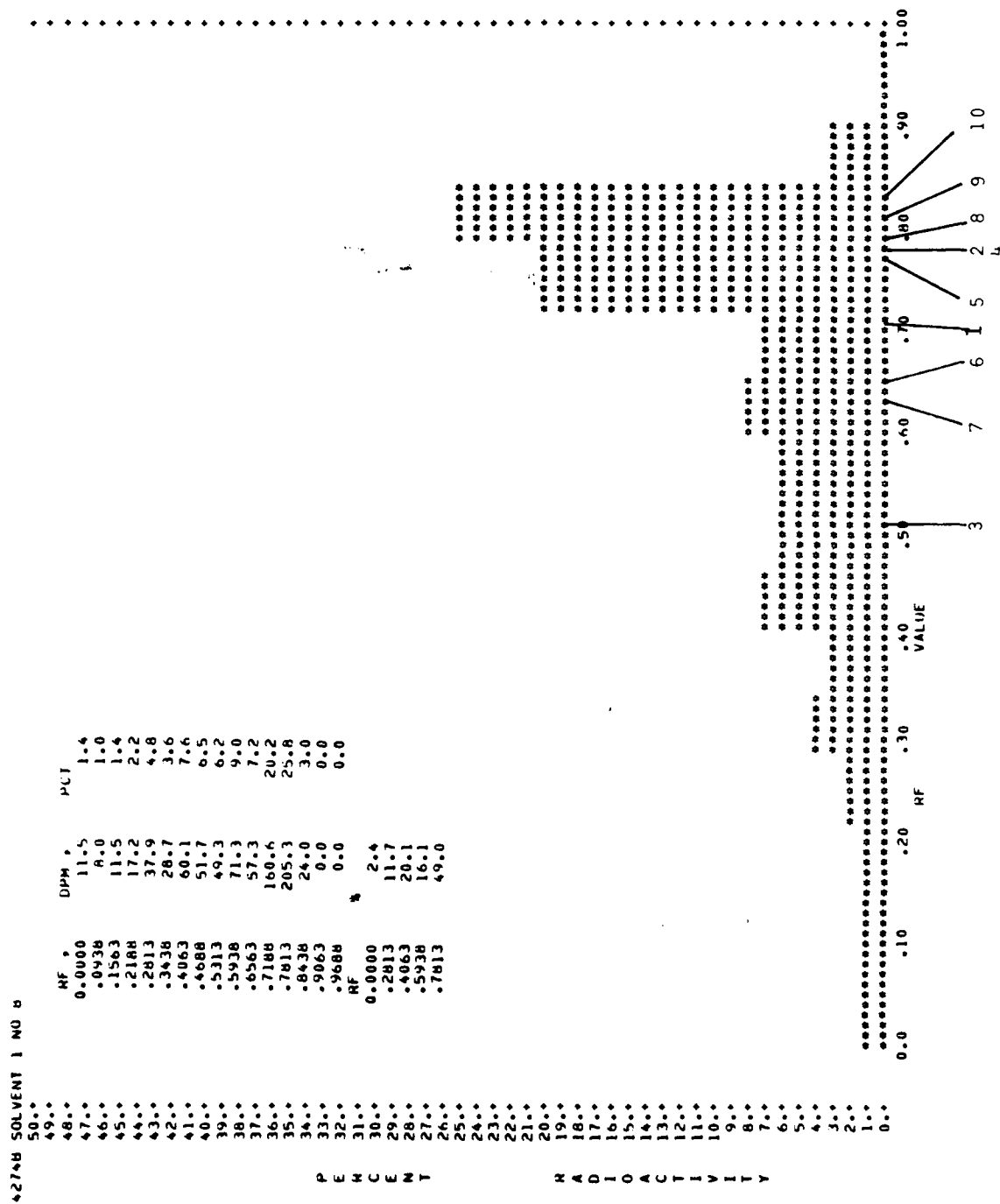


Figure 24-a-I: Rabbit, Oral Treatment, Ethyl Acetate Extract, Solvent I

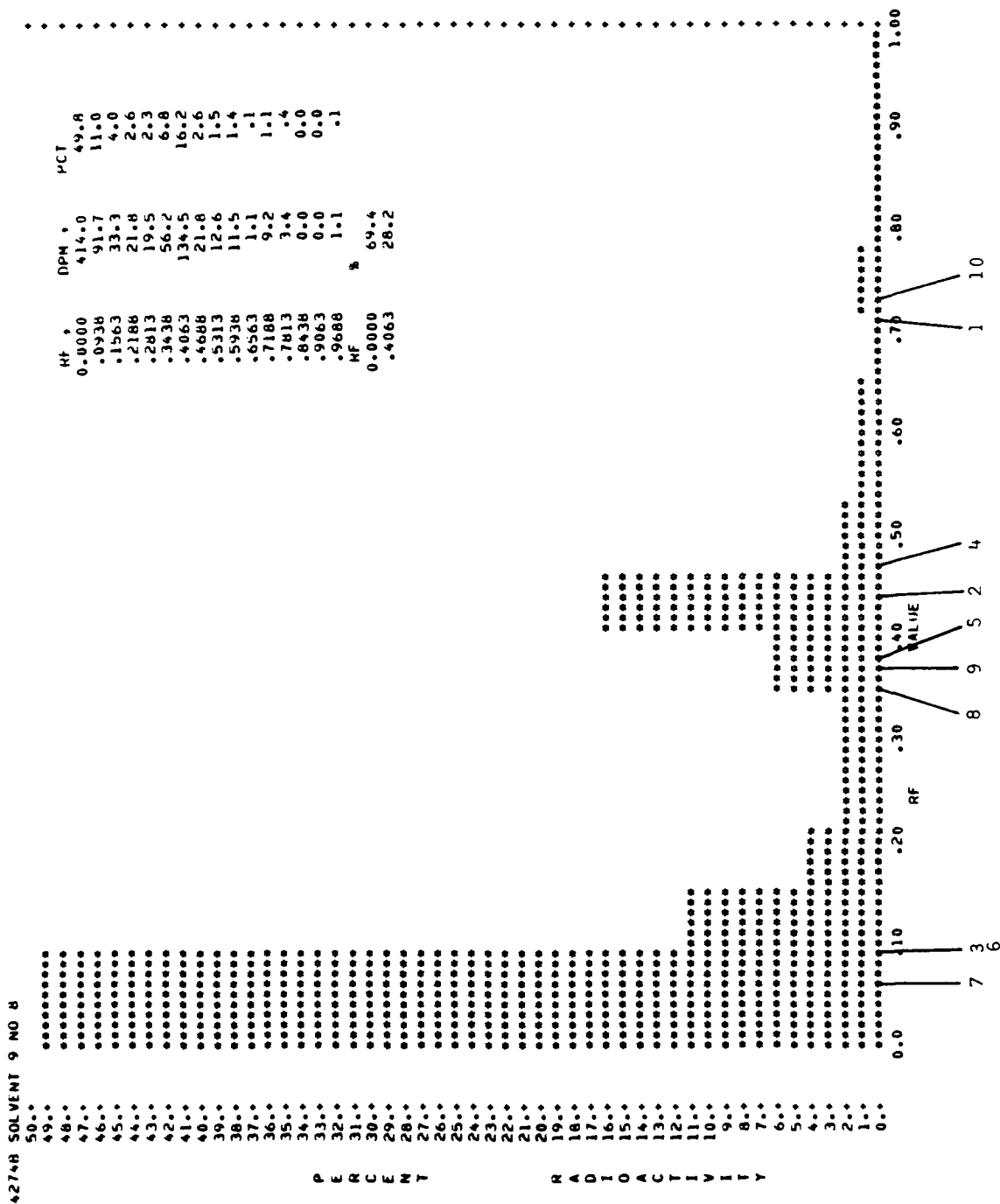


Figure 24-a-IX: Rabbit, Oral Treatment, Ethyl Acetate Extract, Solvent IX

SOLVENT 1 NO 9

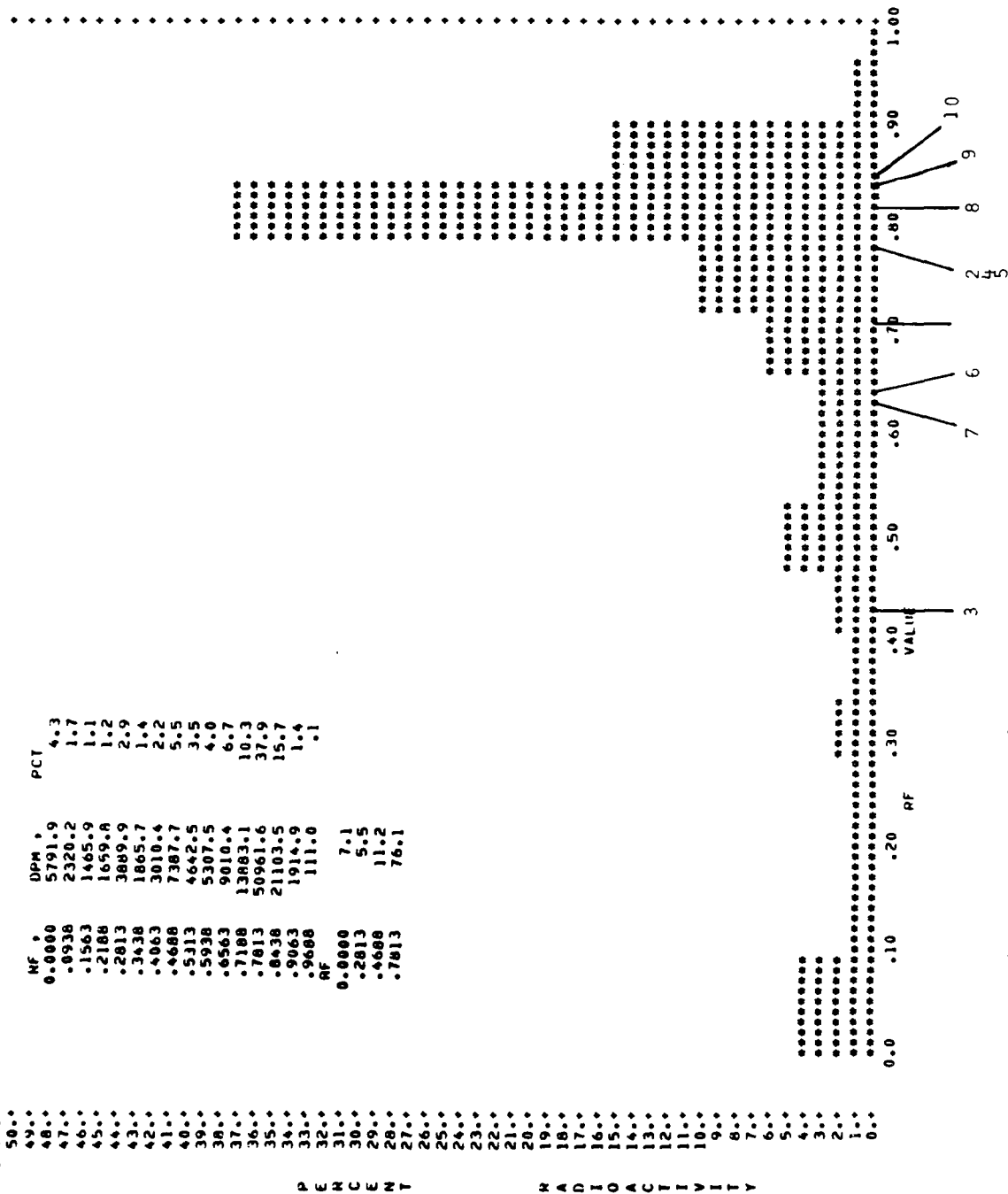


Figure 24-b-I: Dog, Oral Treatment, Ethyl Acetate Extract, Incubation With Water, Solvent I

SOLVENT 4 NO 9

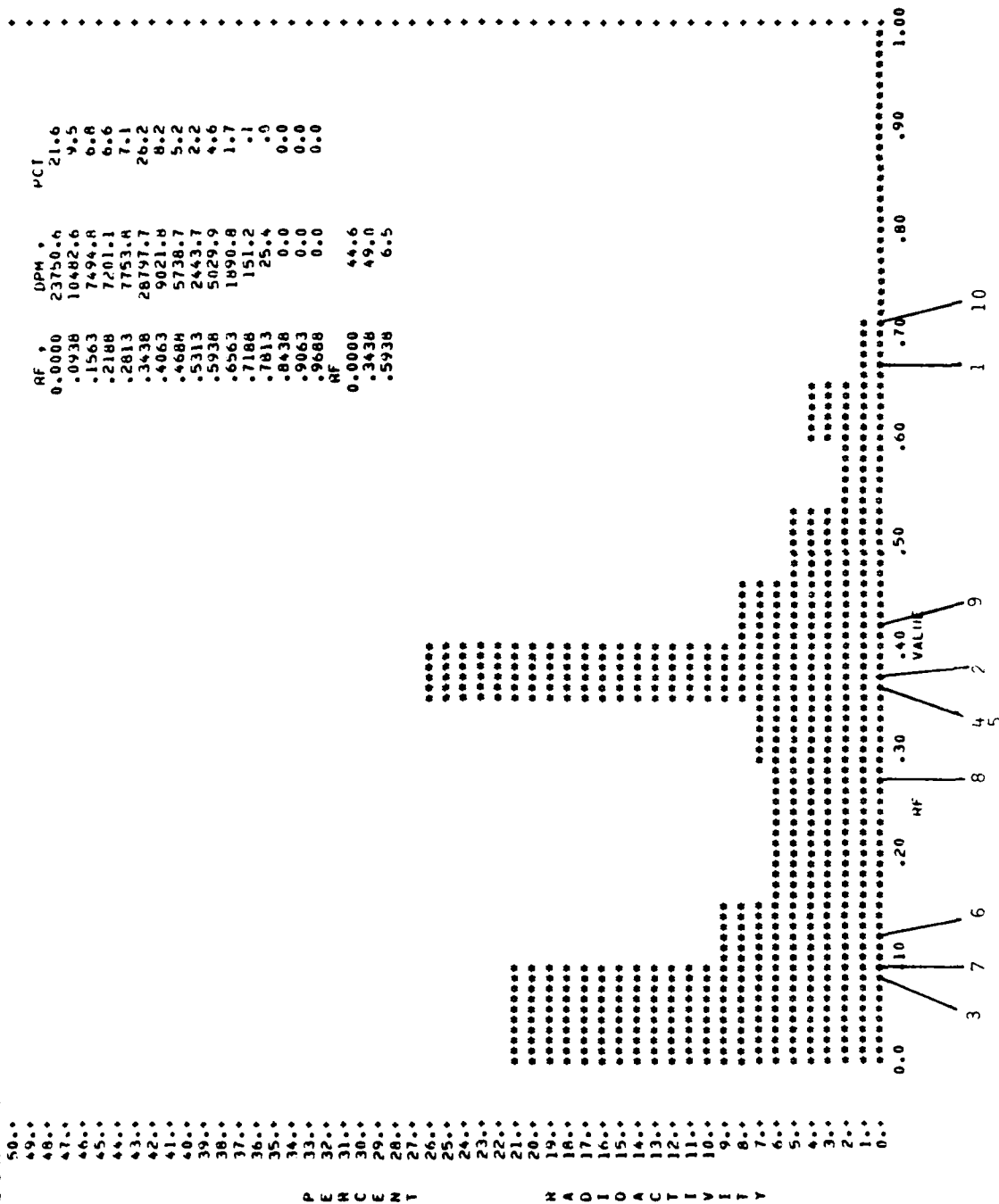


Figure 24-b-IX: Dog, Oral Treatment, Ethyl Acetate Extract, Incubation With Water, Solvent IX

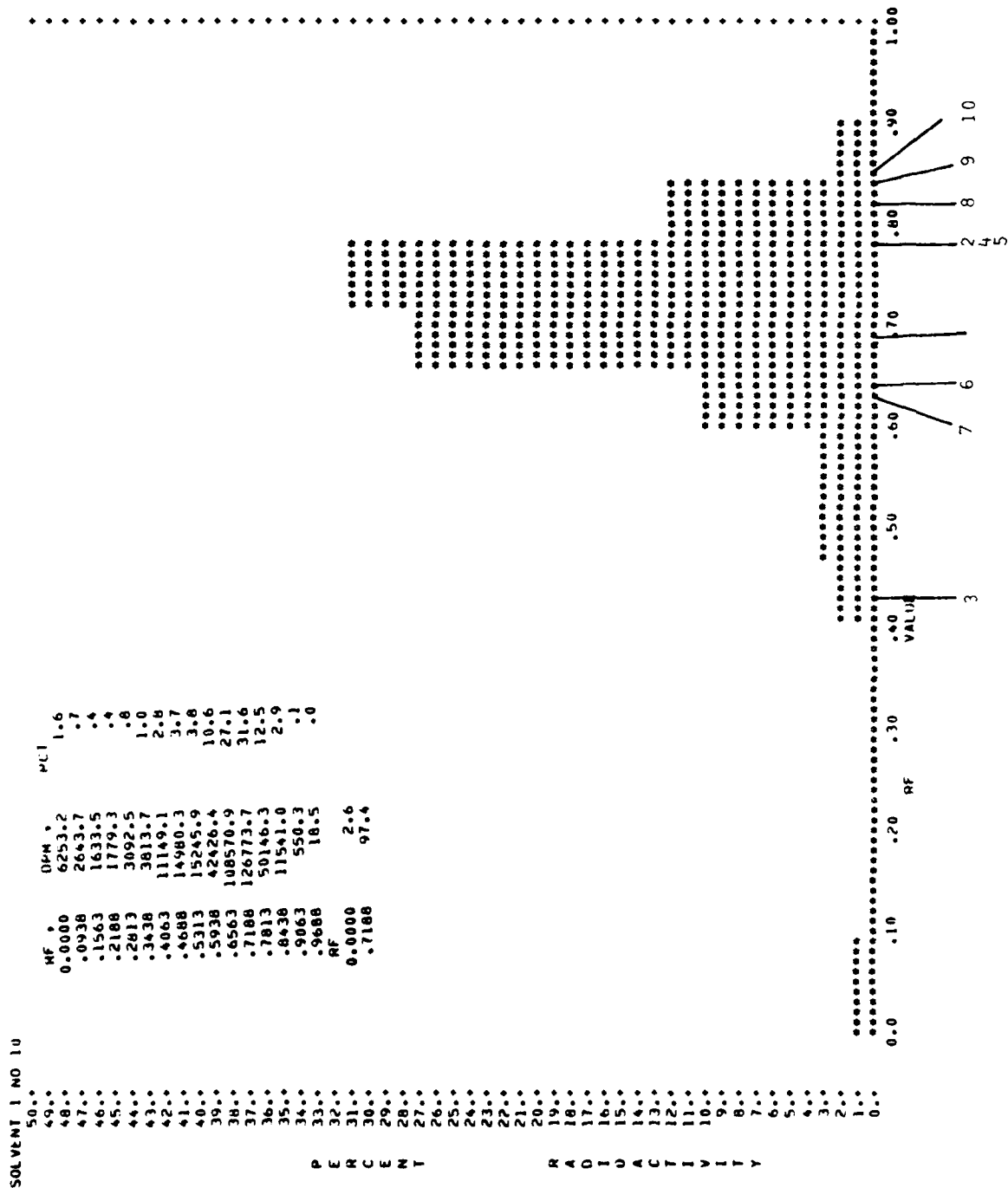


Figure 24-c-I: Dog, Oral Treatment, Ethyl Acetate Extract, Incubation With β -Glucuronidase, Solvent I

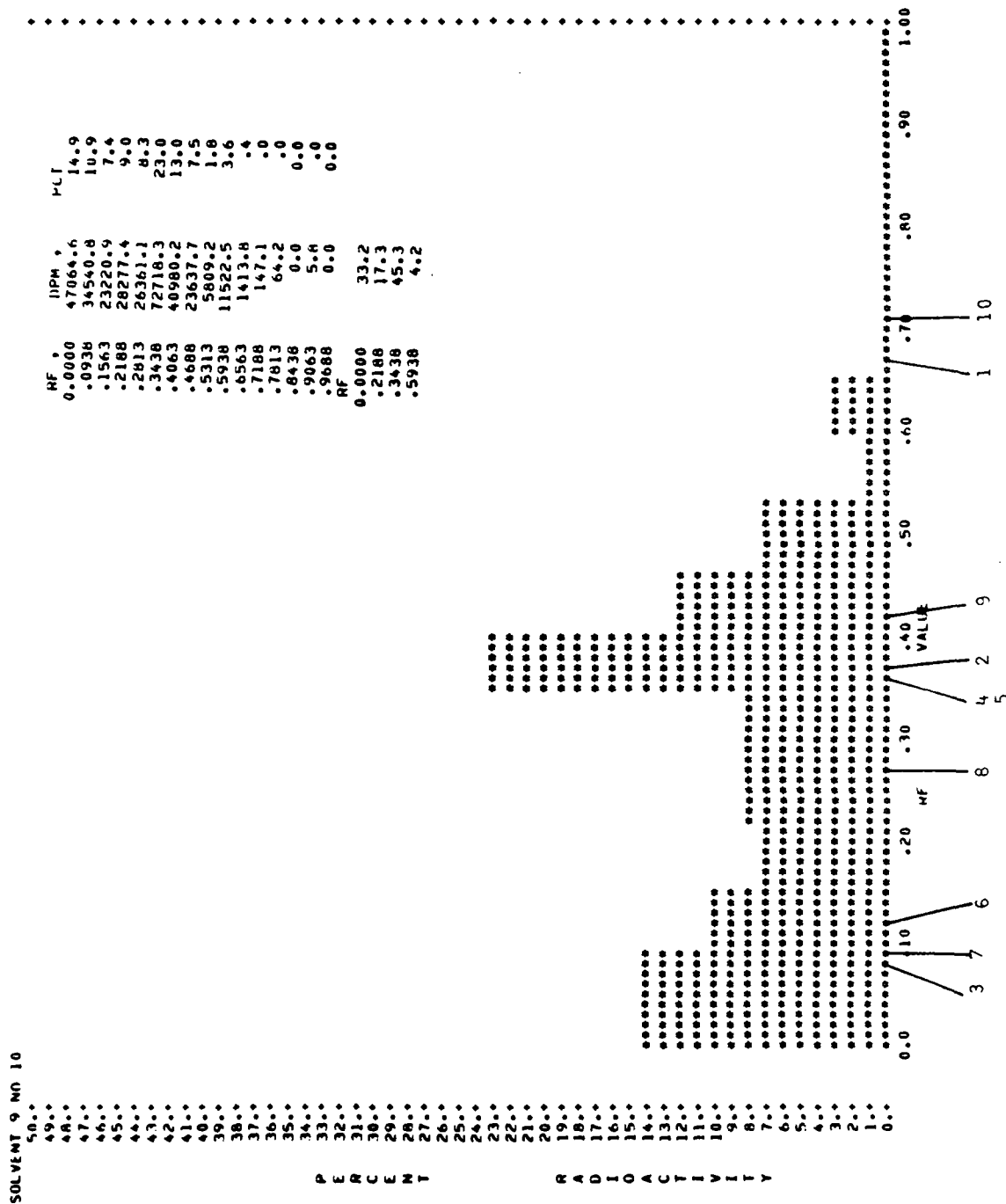


Figure 24-c-IX: Dog, Oral Treatment, Ethyl Acetate Extract, Incubation With β -Glucuronidase, Solvent IX

4274H SOLVENT I NO 13

WT.	DPM	RF
50.00	51.6	.1
49.00	17.2	.1
48.00	.0938	.1
47.00	.1563	.2
46.00	.2188	.2
45.00	.2813	.3
44.00	.3438	.3
43.00	.4063	.4
42.00	.4688	.4
41.00	.5313	.5
40.00	.5938	.5
39.00	.6563	.6
38.00	.7188	.6
37.00	.7813	.7
36.00	.8438	.7
35.00	.9063	.8
34.00	.9688	.8
33.00	.4688	.9
32.00	.7813	.9
31.00	.4688	.9
30.00	.7813	.9
29.00	.4688	.9
28.00	.7813	.9
27.00	.4688	.9
26.00	.7813	.9
25.00	.4688	.9
24.00	.7813	.9
23.00	.4688	.9
22.00	.7813	.9
21.00	.4688	.9
20.00	.7813	.9
19.00	.4688	.9
18.00	.7813	.9
17.00	.4688	.9
16.00	.7813	.9
15.00	.4688	.9
14.00	.7813	.9
13.00	.4688	.9
12.00	.7813	.9
11.00	.4688	.9
10.00	.7813	.9
9.00	.4688	.9
8.00	.7813	.9
7.00	.4688	.9
6.00	.7813	.9
5.00	.4688	.9
4.00	.7813	.9
3.00	.4688	.9
2.00	.7813	.9
1.00	.4688	.9

P F M C E N T H A D I O A C T I V I T Y

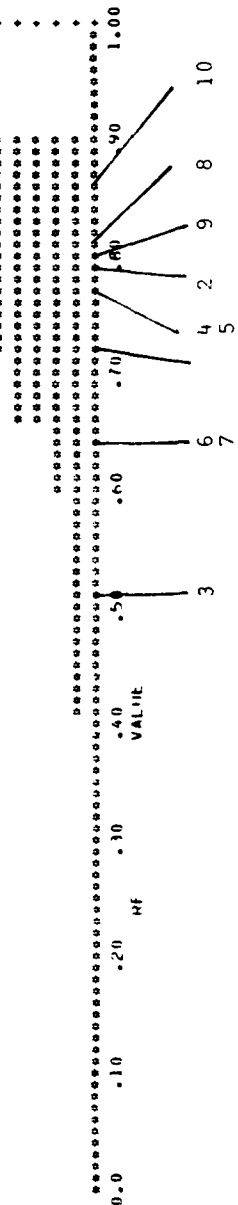


Figure 24-d-I: Dog, Dermal Application, Ethyl Acetate Extract, Incubation With Water, Solvent I

42748 SOLVENT 9 NO 13

50.0	MF	0.0000	IPM	3160.7	PC1	14.1
49.0		.0938		1582.8		6.6
48.0		.1563		1169.9		4.9
47.0		.2188		2782.7		11.6
46.0		.2813		1349.5		5.6
45.0		.3438		1941.0		8.1
44.0		.4063		4252.0		17.7
43.0		.4688		1995.4		8.3
42.0		.5313		1715.6		7.1
41.0		.5938		1899.3		7.9
40.0		.6563		1334.1		5.5
39.0		.7188		560.1		2.3
38.0		.7813		270.6		1.1
37.0		.8438		39.3		.2
36.0		.9063		3.5		.0
35.0		.9688		2.3		.0
34.0	MF	0.0000		24.6		
33.0		.2188		17.2		
32.0		.4063		41.2		
31.0		.5938		17.1		

P E R C E N T

R A D I O

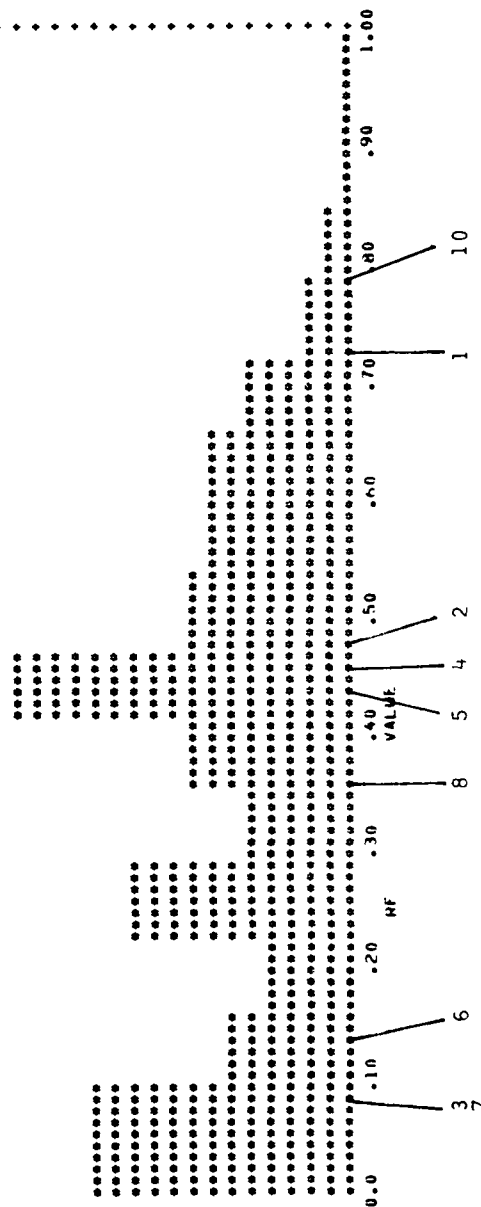


Figure 24-d-IX: Dog, Dermal Application, Ethyl Acetate Extract, Incubation With Water, Solvent IX

HF.	UPM.	PLI
U-0000	116.8	.1
.093B	99.4	.1
.1563	63.1	.2
.2188	70.4	.4
.2813	137.4	.6
.3438	216.7	1.2
.4063	418.5	1.6
.4688	568.2	2.9
.5313	1758.3	4.9
.5938	4774.2	17.0
.6563	14213.3	34.3
.7188	7000.0	19.3
.7813	1423.2	3.9
.8438	303.9	.8
.9063	20.7	.1
.9688	2.7	.0
HF	%	
	99.2	
	6563	

PERCENT RADIOACTIVITY

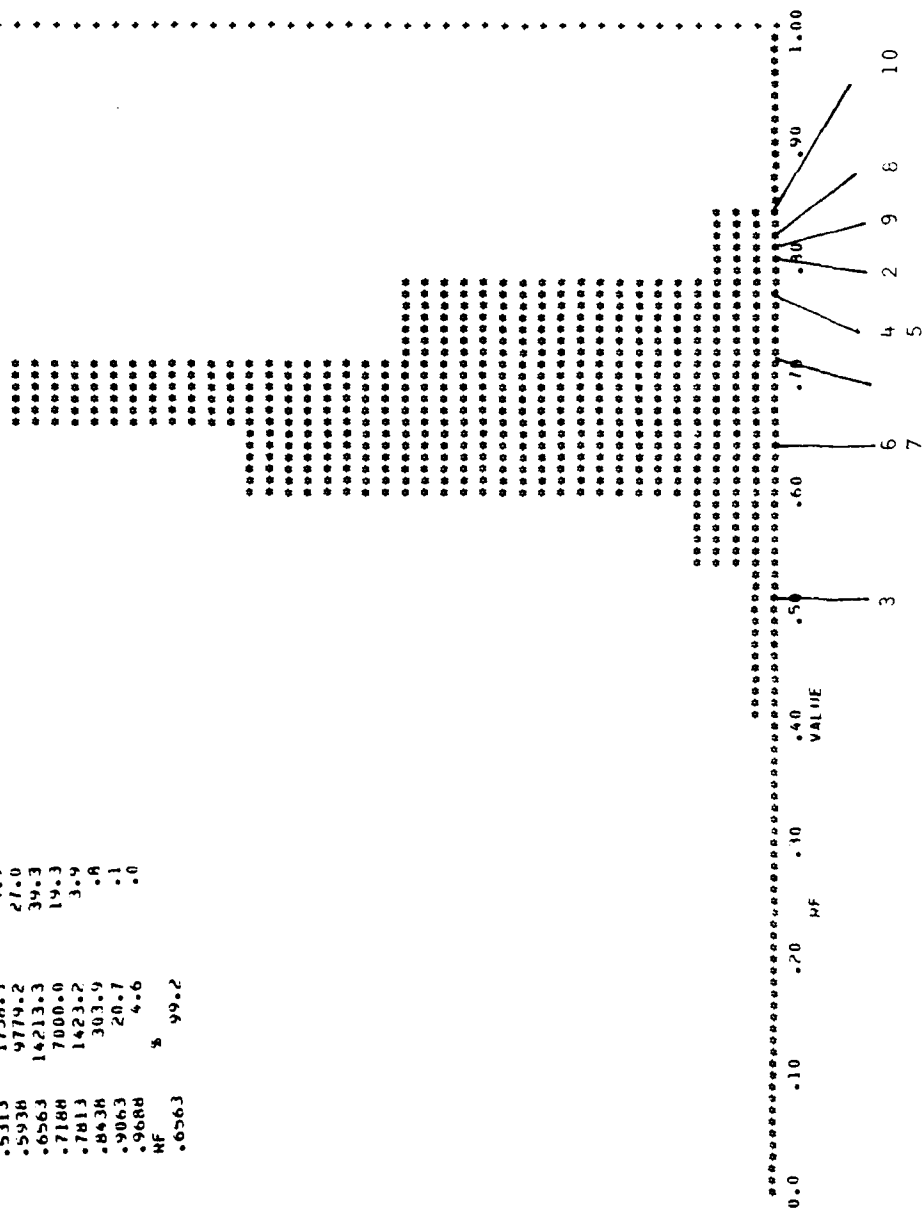


Figure 24-e-I: Dog, Dermal Application, Ethyl Acetate Extract, Incubation With β -Glucuronidase, Solvent I

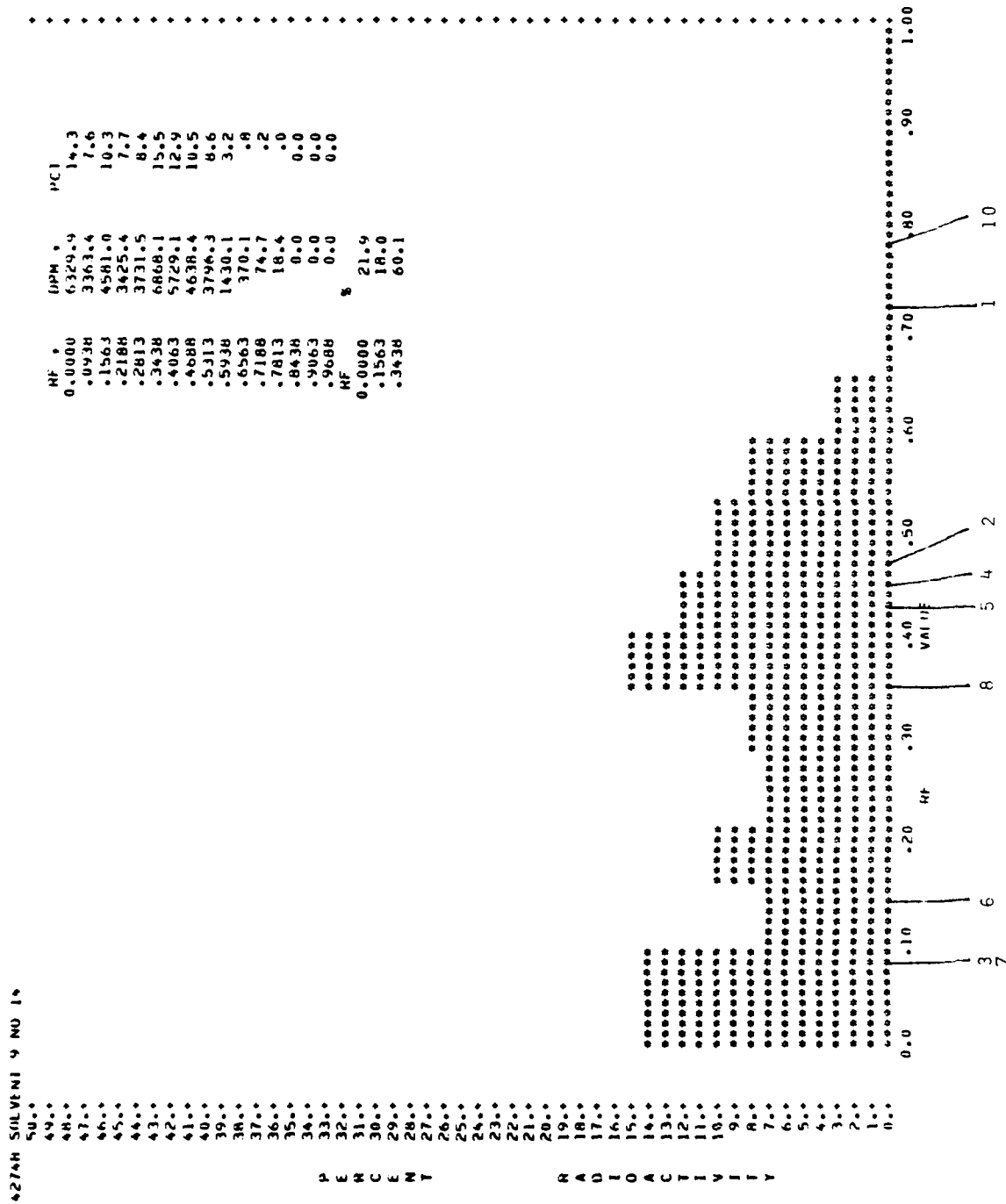


Figure 24-e-IX: Dog, Dermal Application, Ethyl Acetate Extract, Incubation With β -Glucuronidase, Solvent IX

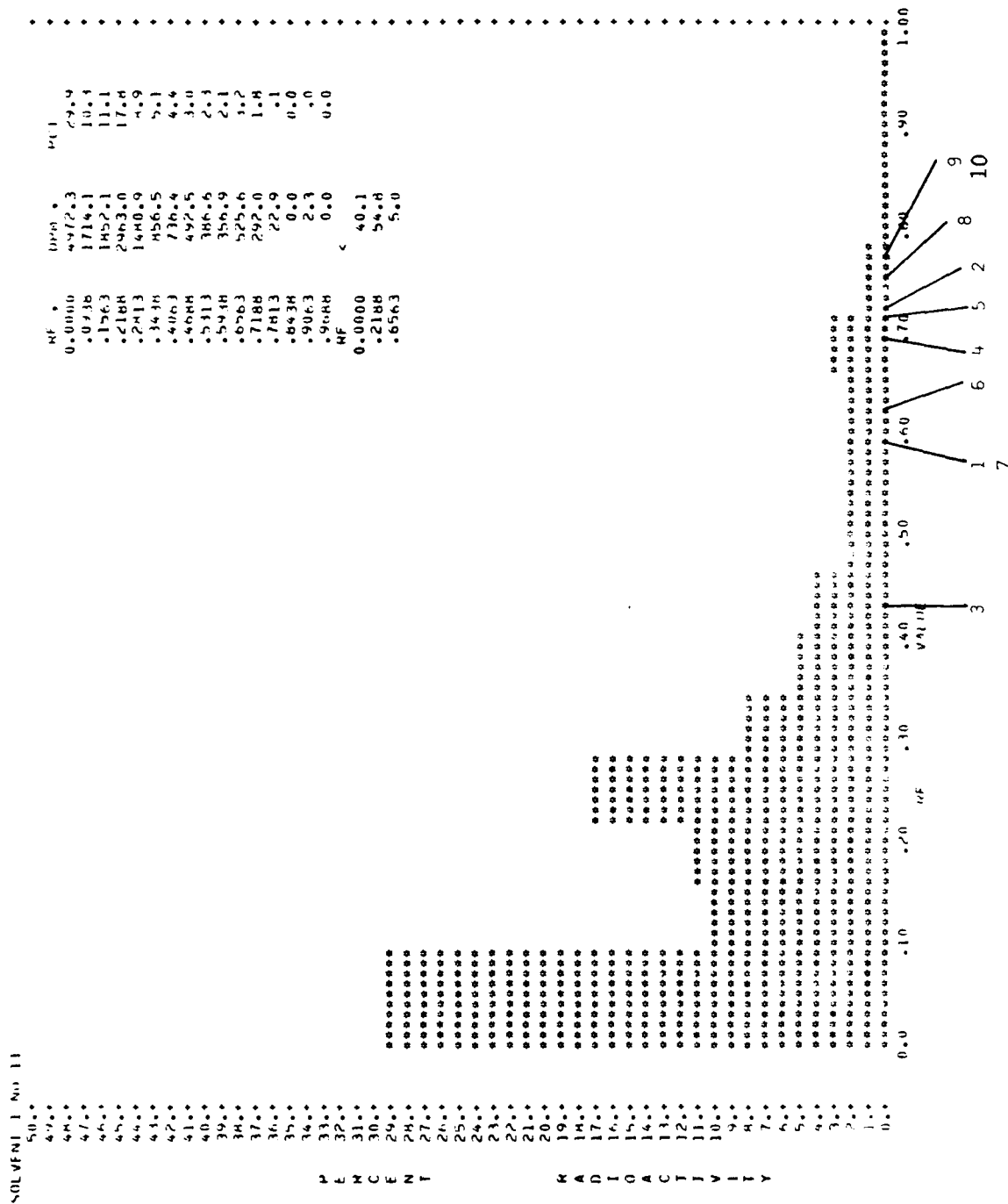


Figure 24-f-I: Dog, Dermal Application, Aqueous Extract, Solvent I

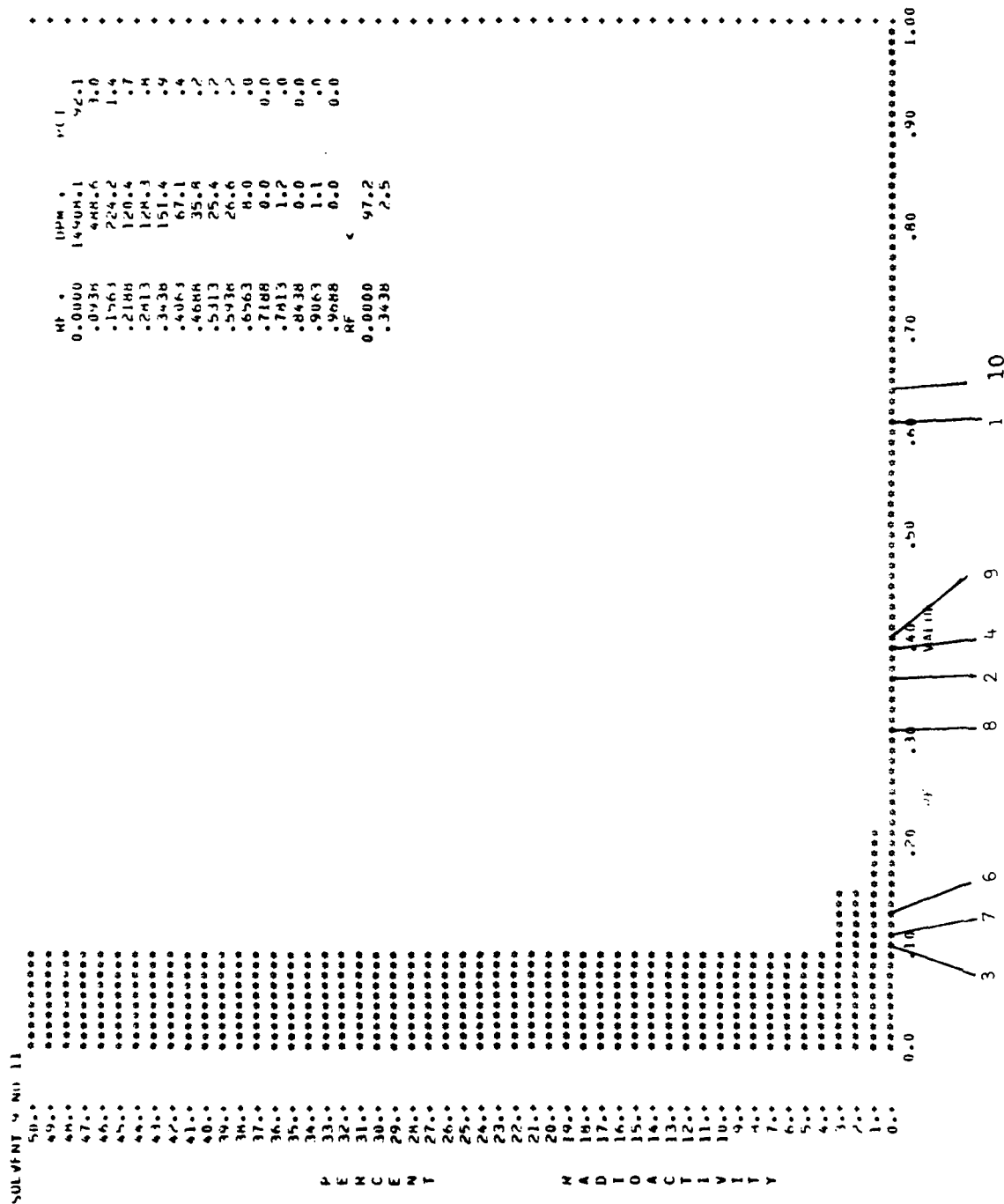


Figure 24-f-IX: Dog, Dermal Application, Aqueous Extract, Solvent IX

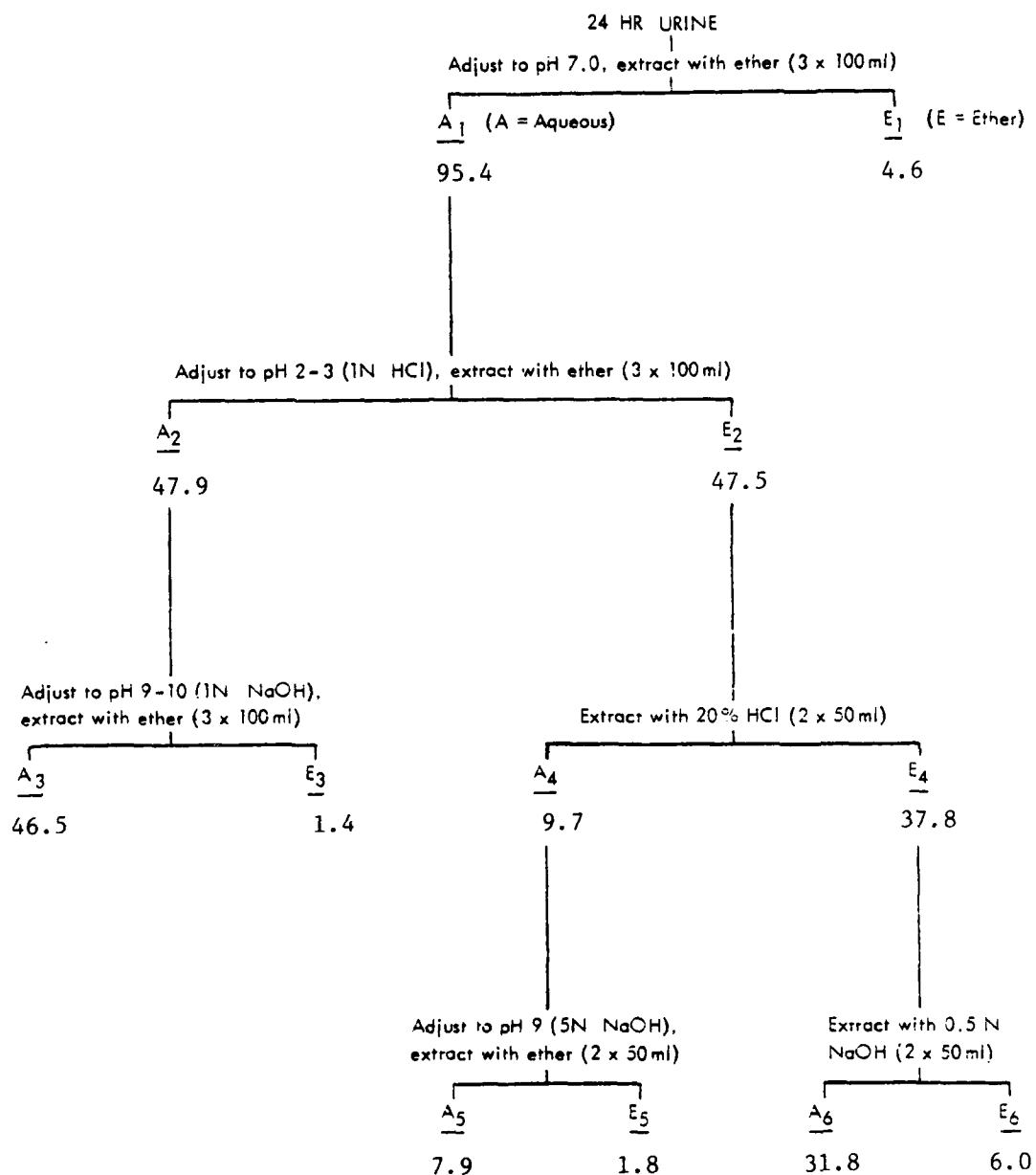


Figure 25: Fractionation of 24-Hr Urine Obtained from Rats Treated Orally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 26, E₁-E₆: TLC of Ether-Extractable Products Obtained from 24-hr Urine of Rats Treated Orally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 1:1:1; Solvent IX, toluene:acetic acid, 4:1. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 26 follows

SOLVENT I NO RAT FI

50.0
49.0
48.0
47.0
46.0
45.0
44.0
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

P
F
R
C
E
N
T

RF .
0.0000
0.0334
0.1563
0.2188
0.2813
0.3438
0.4063
0.4688
0.5313
0.5938
0.6563
0.7188
0.7813
0.8438
0.9063
0.9688
NF
0.0000
5.0
4.1
55.6
35.1
0.0

10PM .

100

1.2
1.0
2.1
2.0
2.8
3.0
4.3
4.9
10.1
13.7
12.8
21.1
11.7
1.4
5
0.0

R
A
D
I
U
A
C
I
T
Y

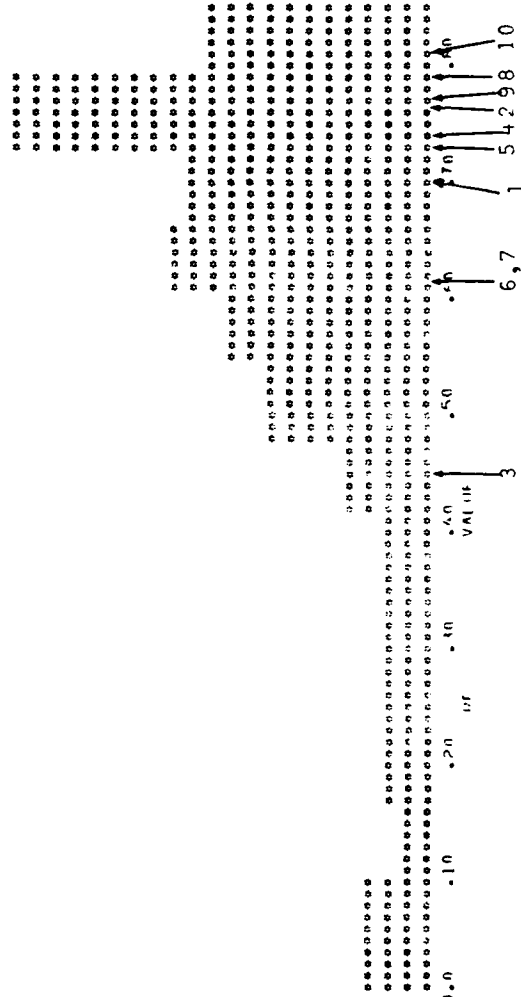


Figure 26-E1: Solvent I

2748 MAY 25 1978 TALLAHASSEE SOLVENT 9 NO DATA

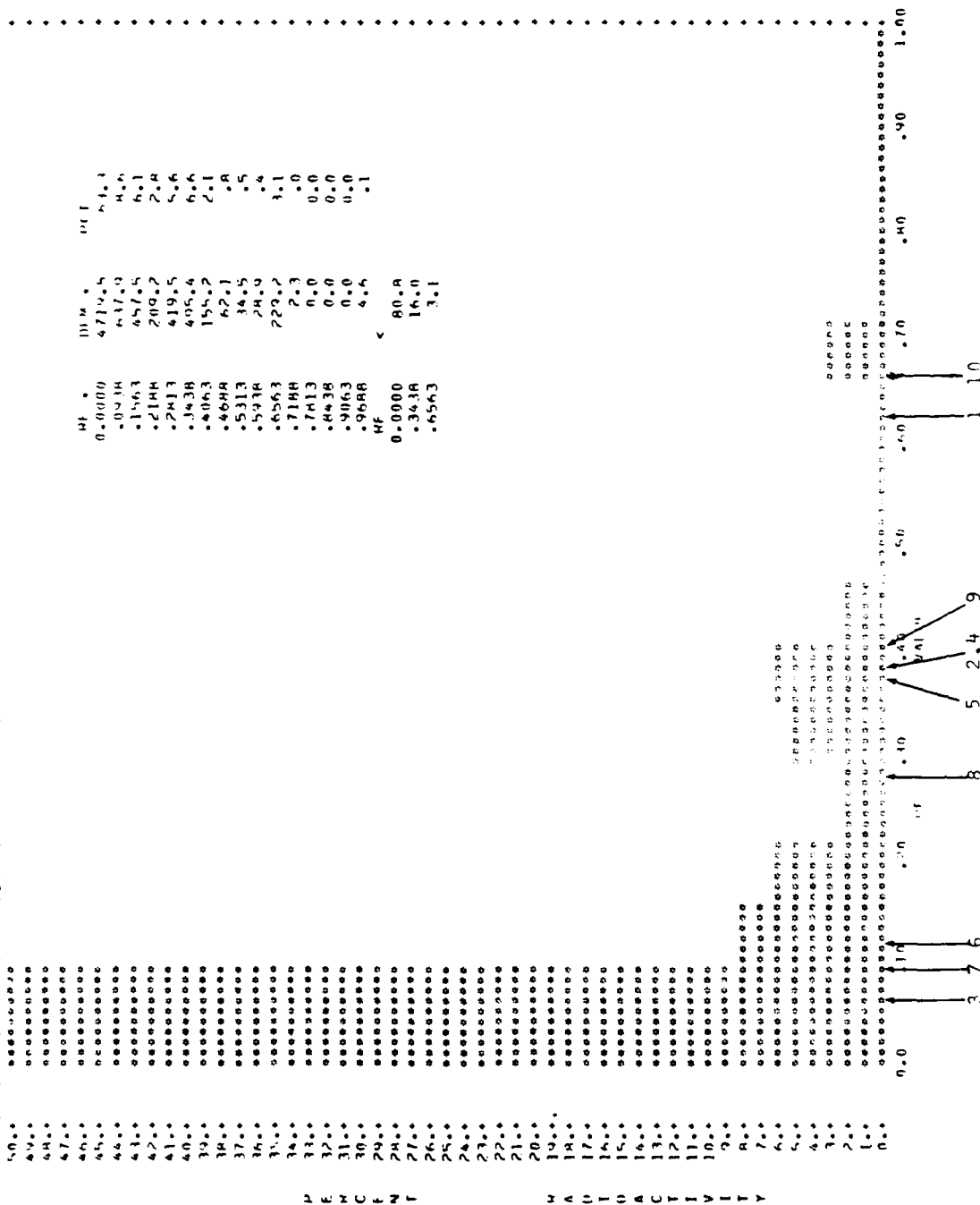


Figure 26-E1: Solvent IX

SOLVENT 1 NO PAT F2

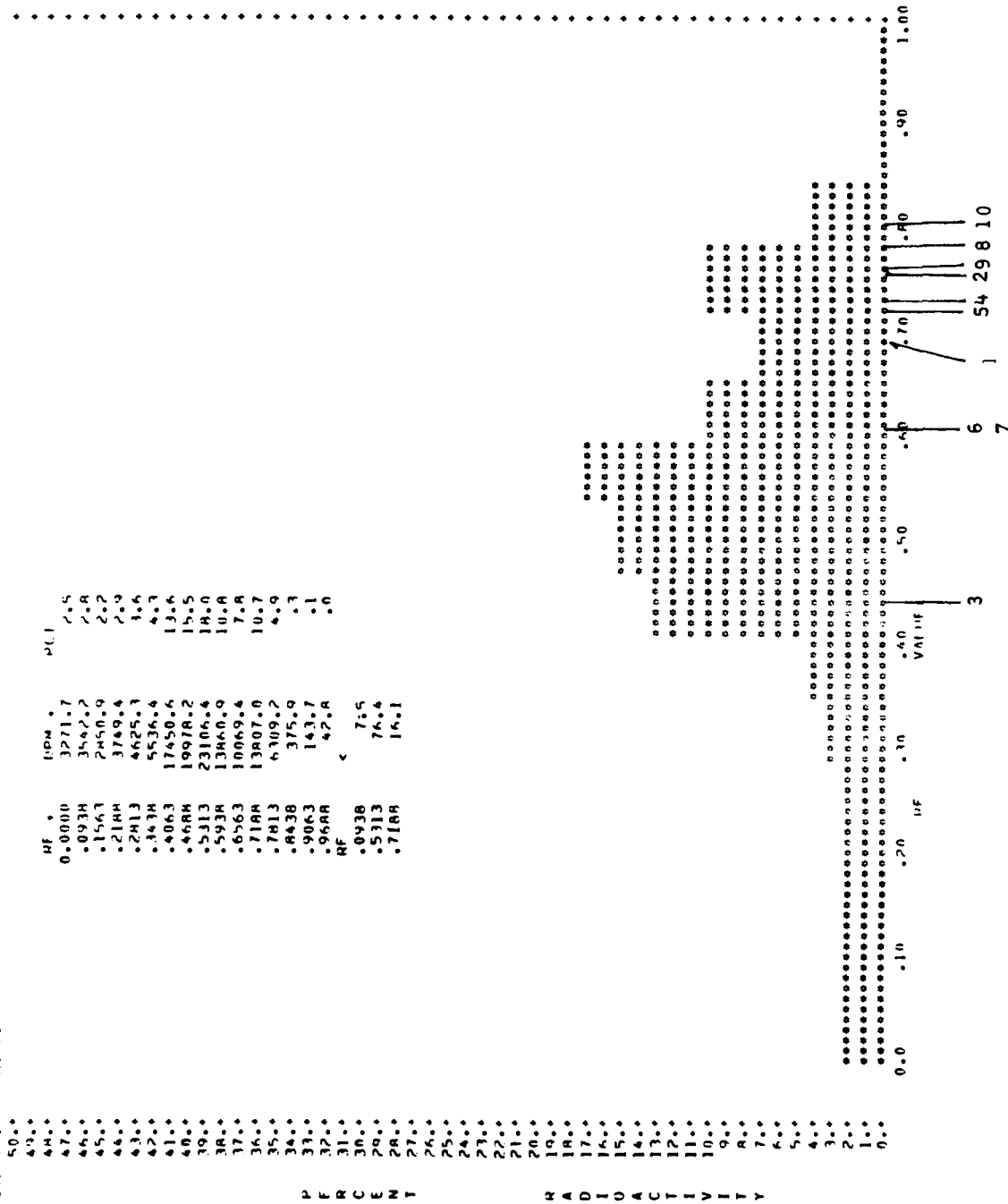


Figure 26-E2: Solvent I

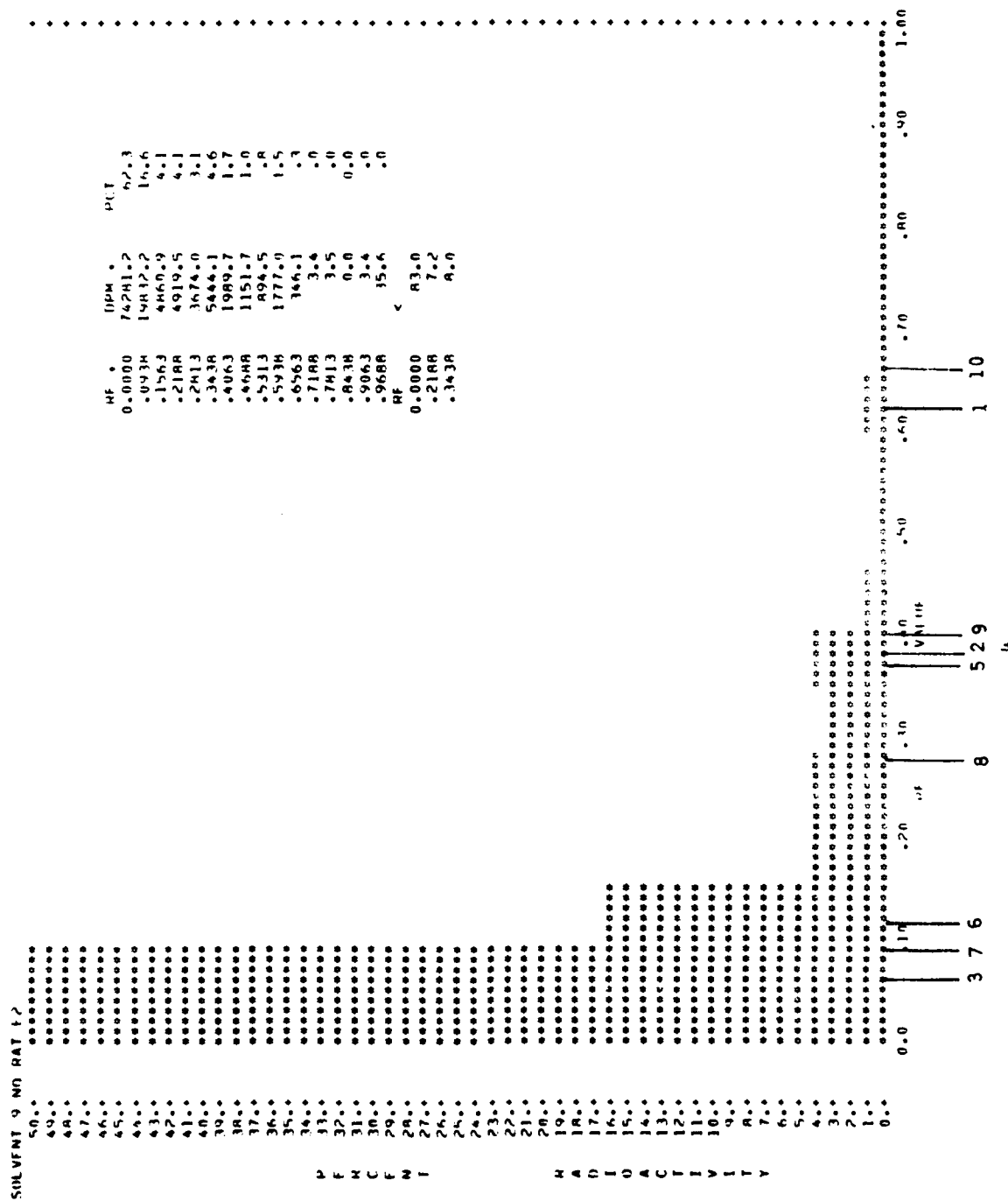


Figure 26-E2: Solvent IX

SOLVENT 1 NO MAT F3

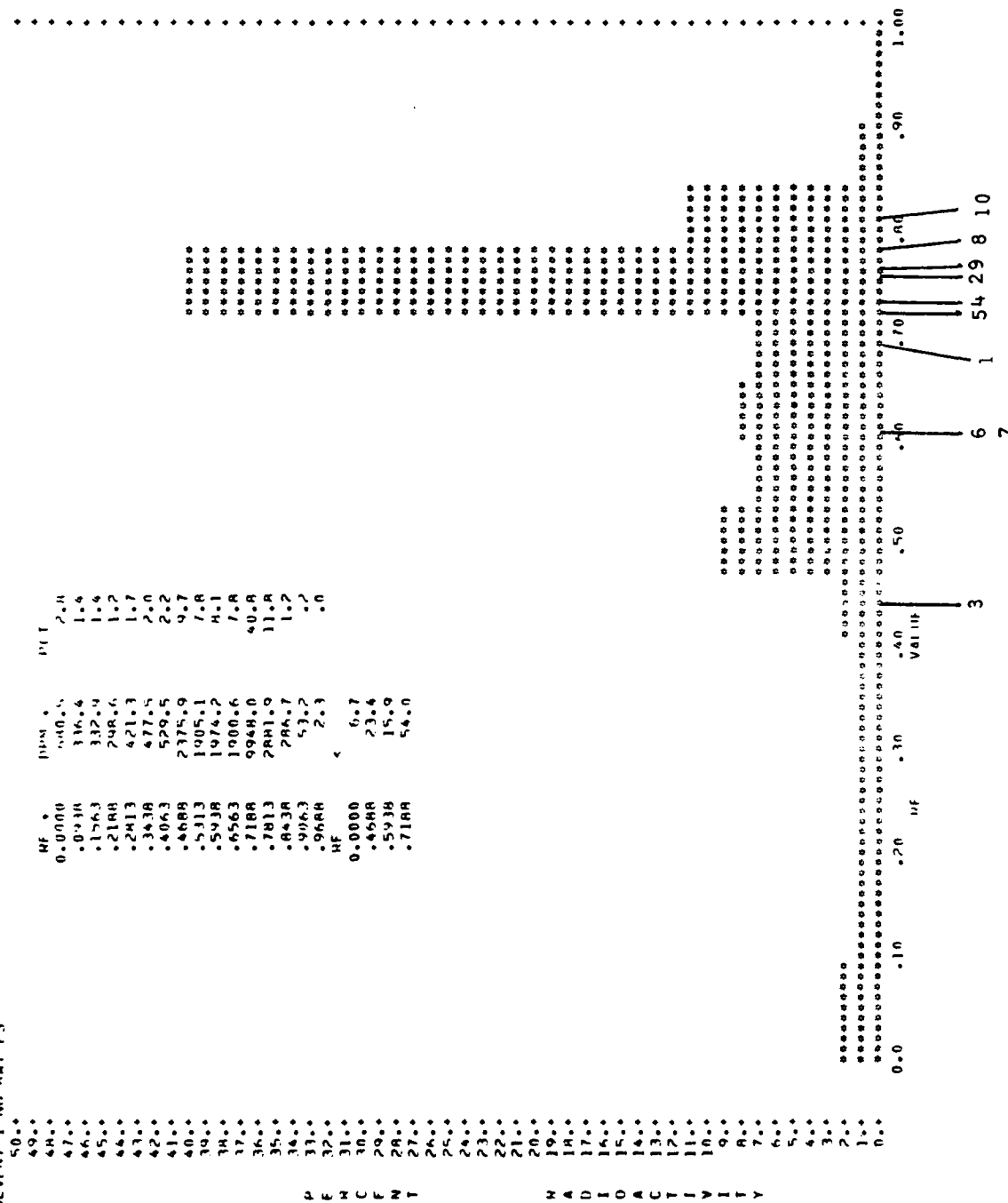


Figure 26-E3: Solvent I

SOLVENT 9 NO RAT F3

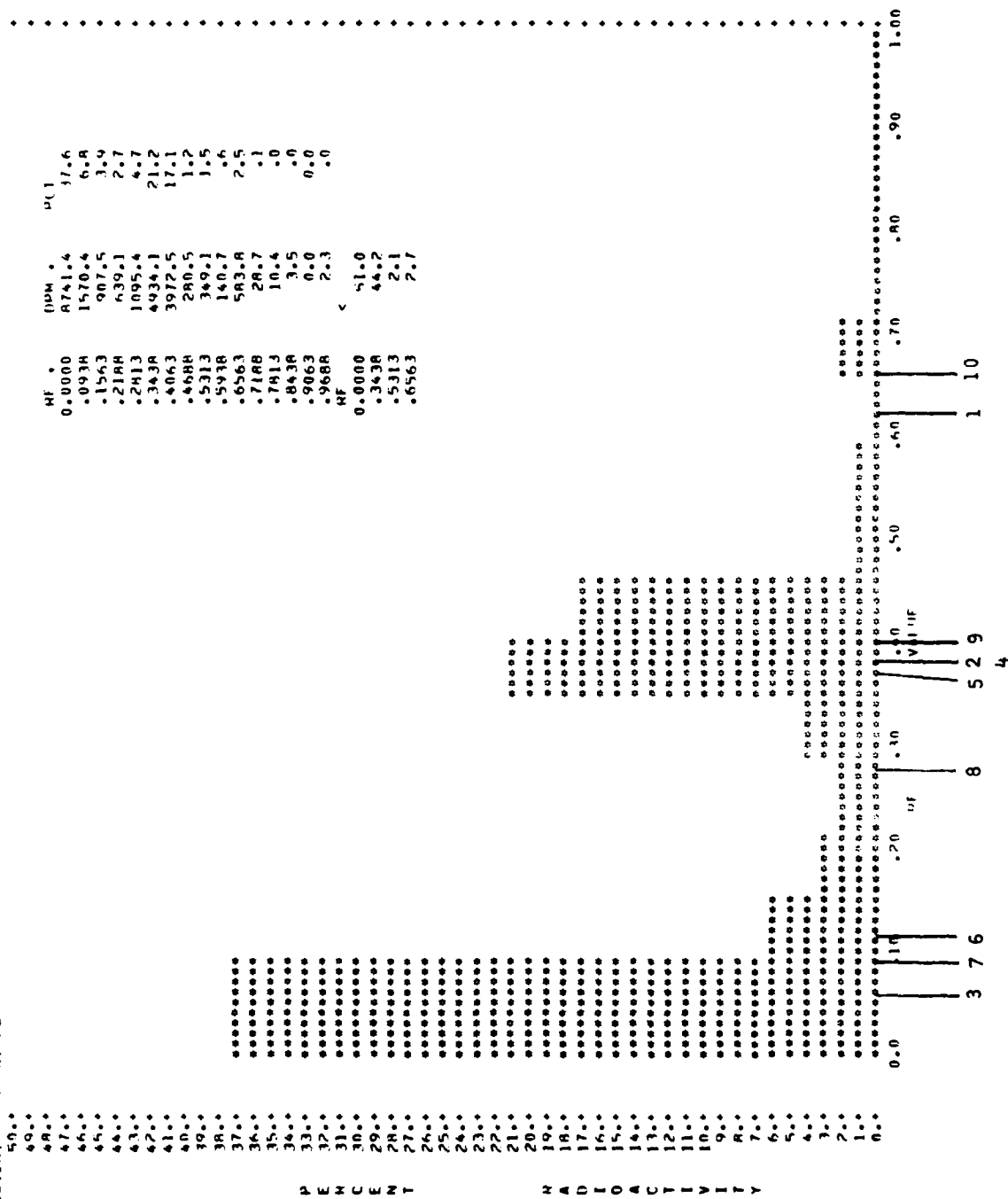


Figure 26-E3: Solvent IX

SOLVENT I NO PAT F*

50.0
49.0
48.0
47.0
46.0
45.0
44.0
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

P E H C F N T

R A D I U A C T I V I T Y

HF
0.0000
.093M
.1563
.2188
.2813
.3438
.4063
.4688
.5313
.5938
.6563
.7188
.7813
.8438
.9063
.9688
HF
0.0000
.4688
.7188

10M
2003.5
1731.7
1316.1
1710.6
2259.8
2961.8
3165.8
16860.1
3800.0
8957.5
8865.5
12827.9
11369.2
1302.1
197.7
31.0
5.3
67.2
21.4

PI
2.1
1.8
1.4
1.8
2.4
3.1
12.3
15.7
13.8
9.5
8.5
13.6
12.0
1.4
.6
.0

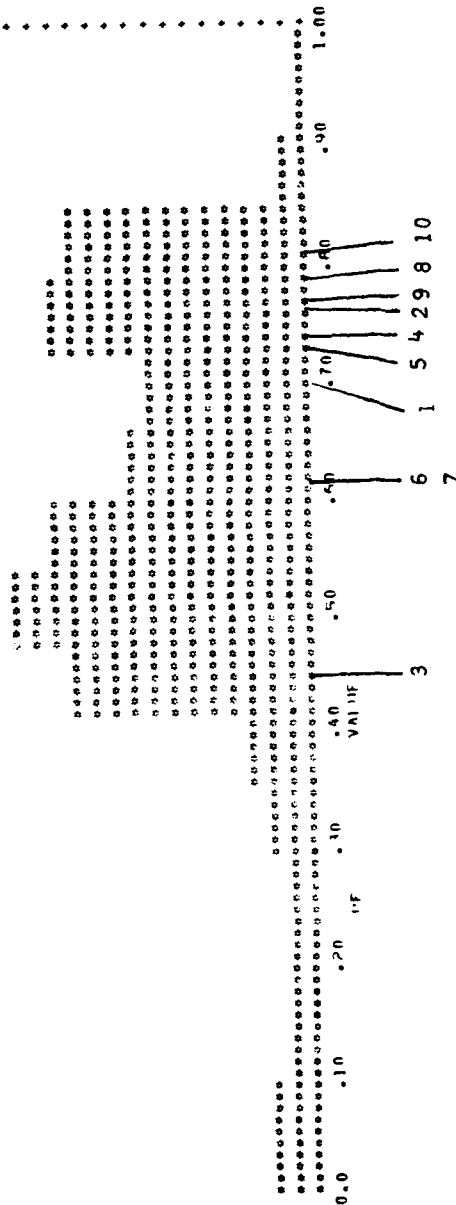
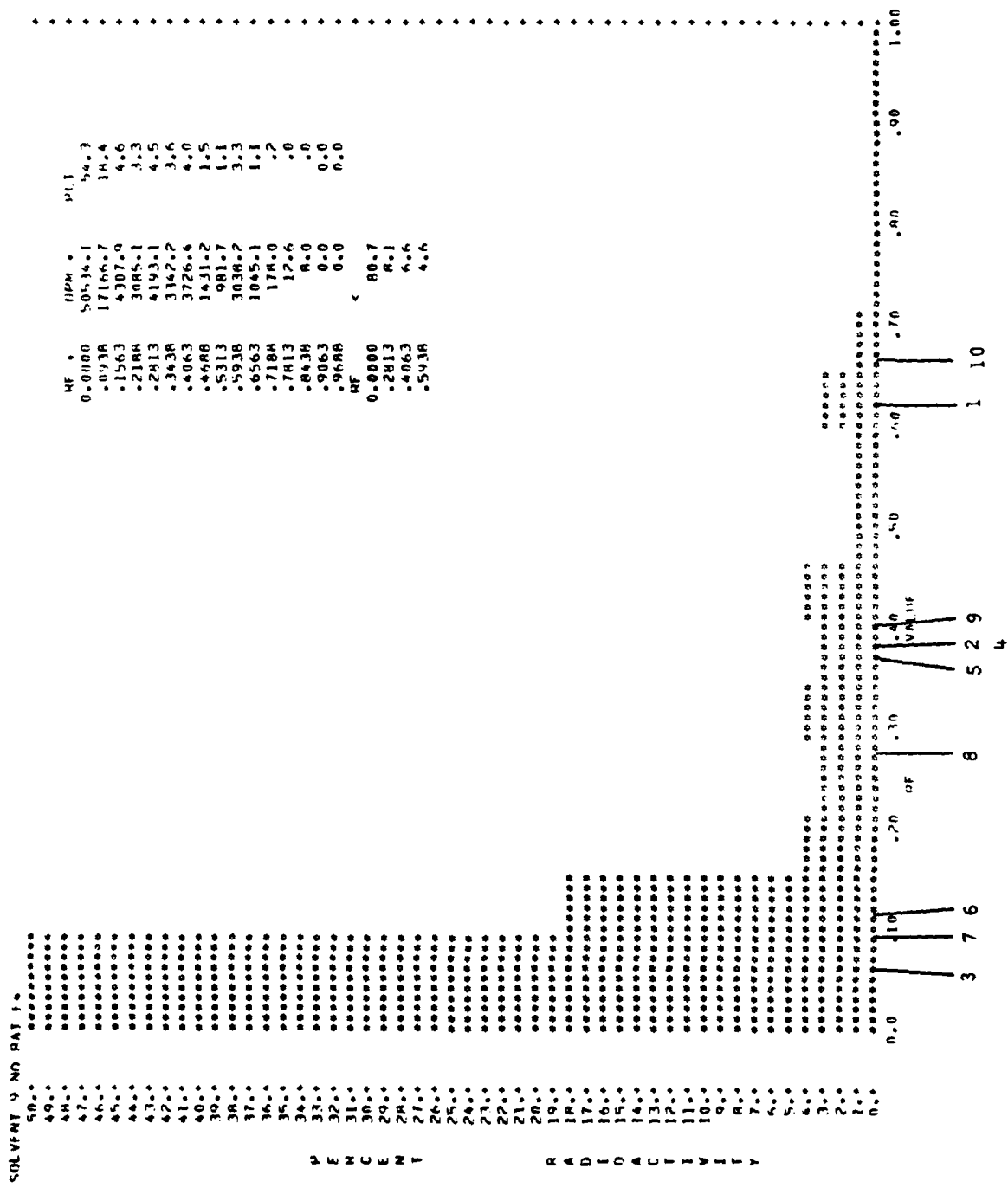
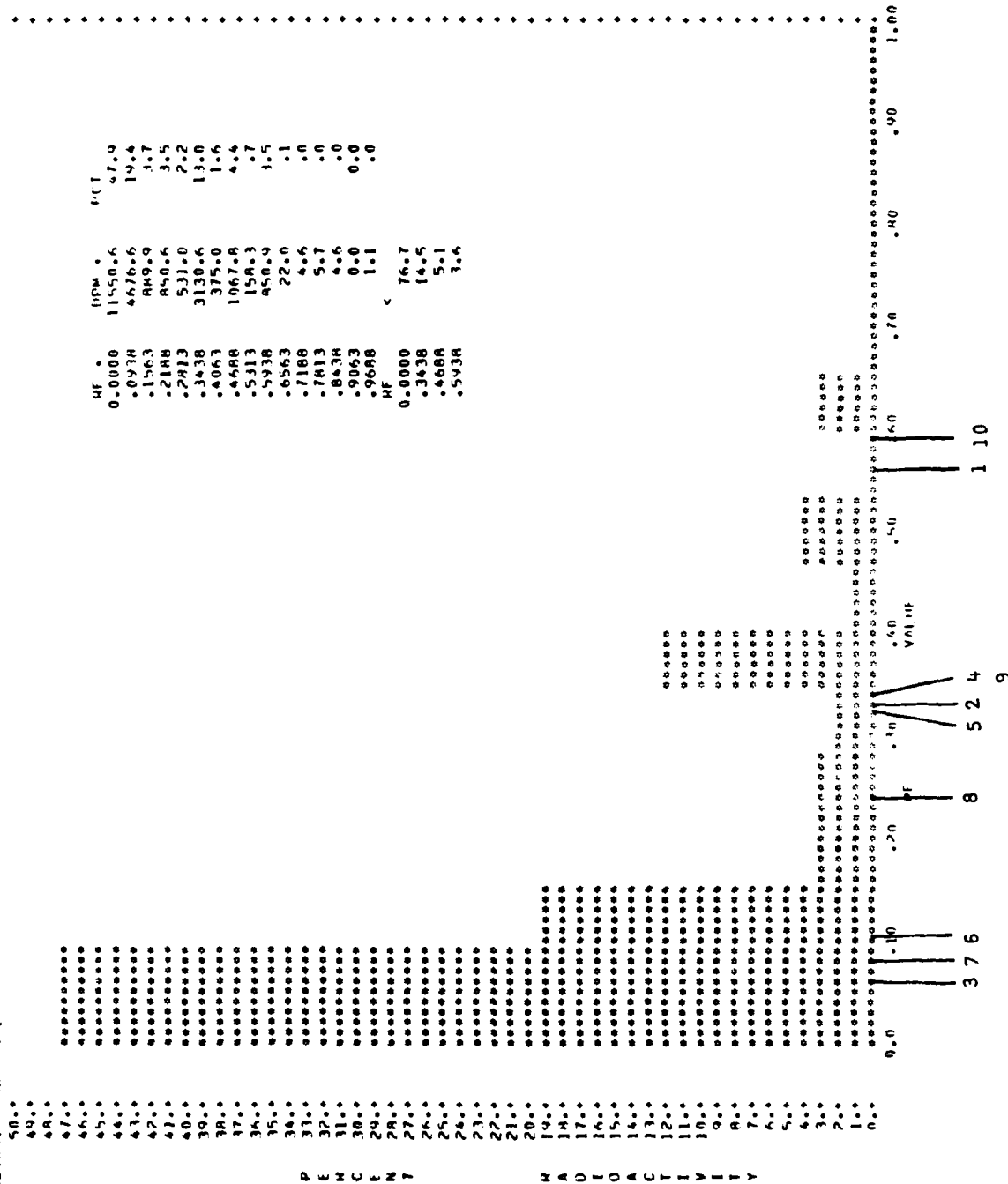


Figure 26-E₄: Solvent I



SOLVENT 9 NO DAT F5



HF	HPM	FFT
0.0000	11550.6	47.9
.0938	4676.6	19.4
.1563	849.9	3.7
.2188	850.6	3.5
.2813	531.0	2.2
.3438	3130.6	13.0
.4063	375.0	1.6
.4688	1067.8	4.4
.5313	158.3	.7
.5938	850.9	1.5
.6563	22.0	.1
.7188	4.6	.0
.7813	5.7	.0
.8438	4.6	.0
.9063	0.0	0.0
.9688	1.1	.0
HF		
0.0000	76.7	
.3438	14.5	
.4688	5.1	
.5938	3.4	

Figure 26-E5: Solvent IX

SOLVENT I NO RAT 14

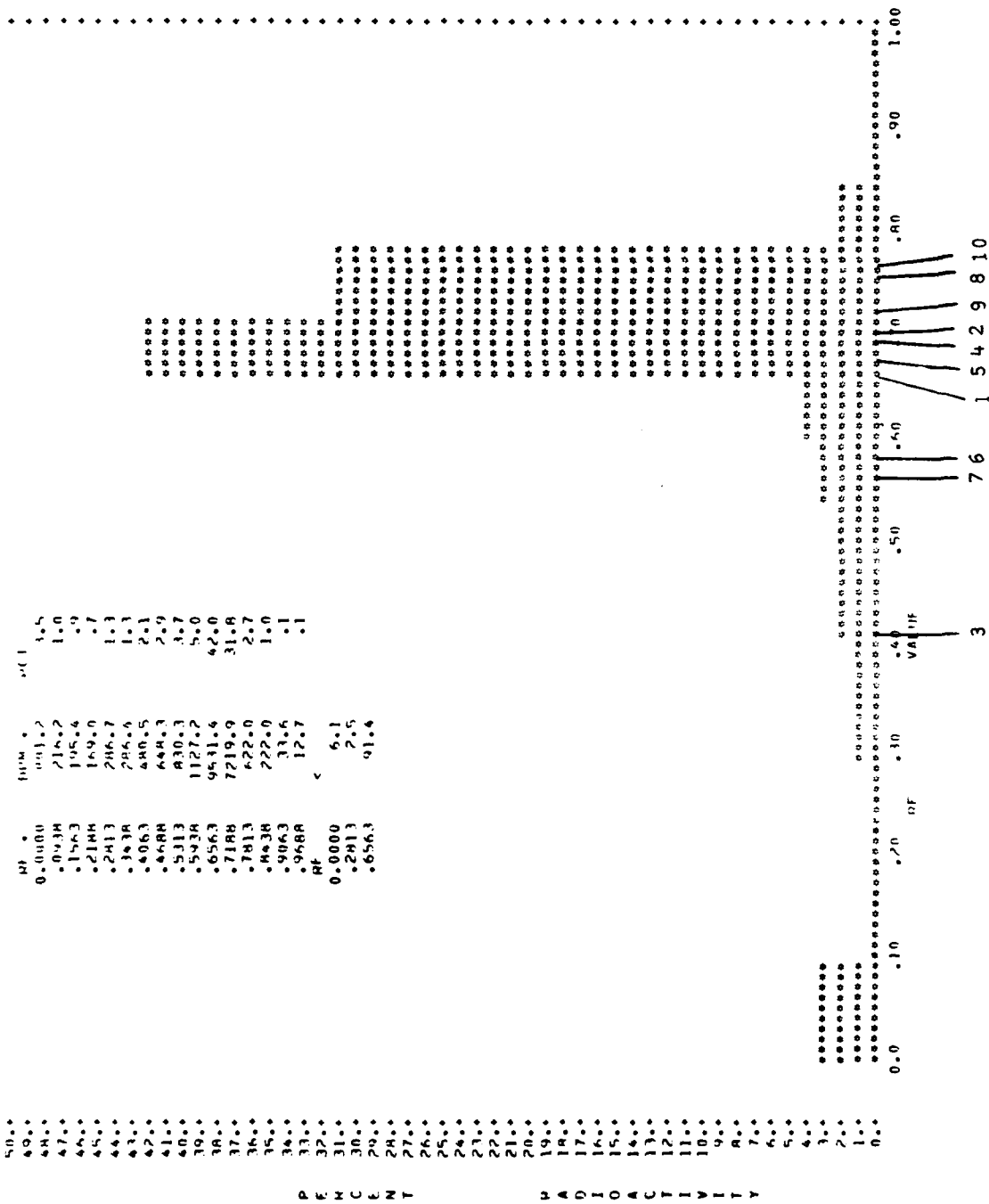


Figure 26-E6: Solvent I

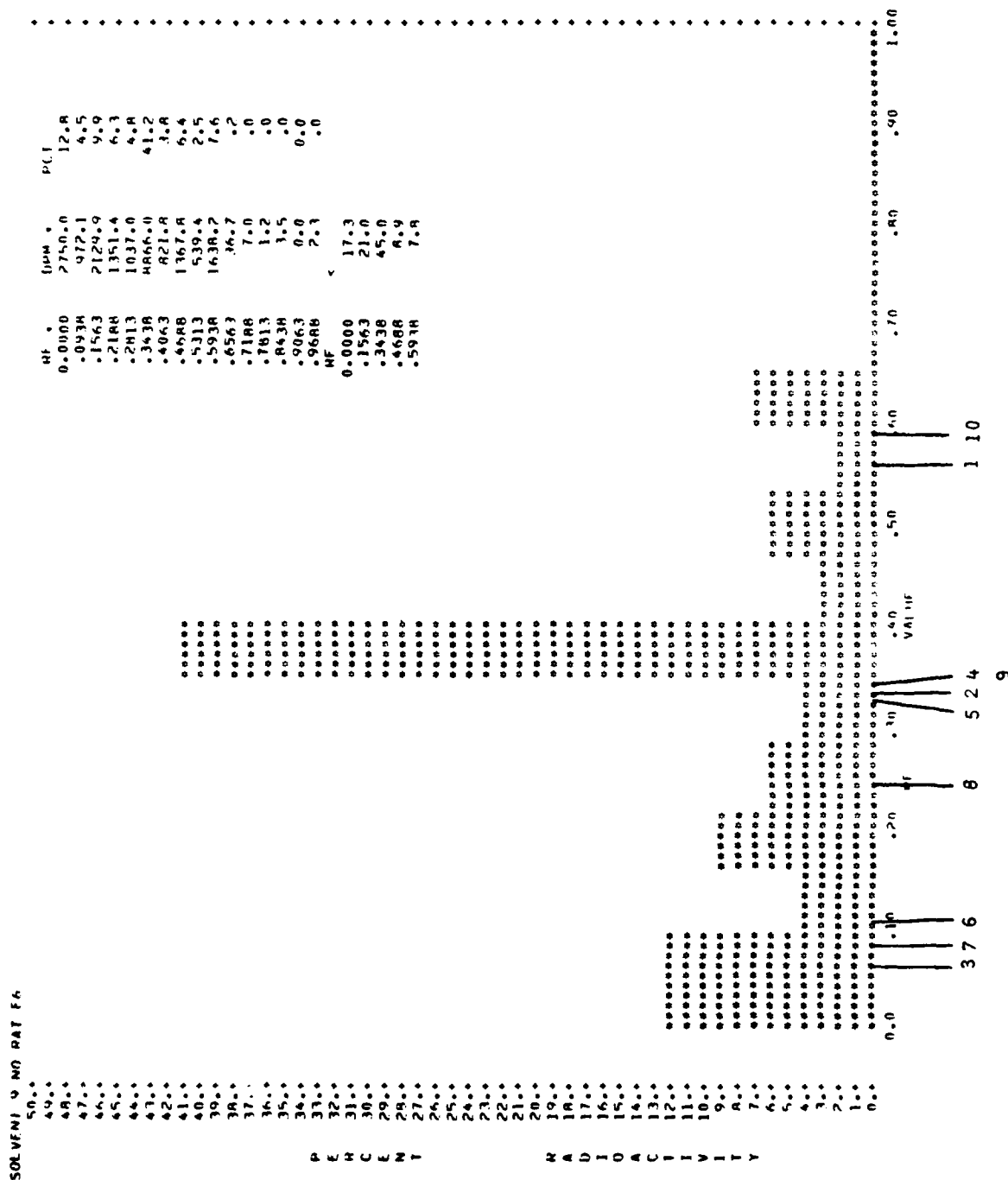


Figure 26-E₆: Solvent IX

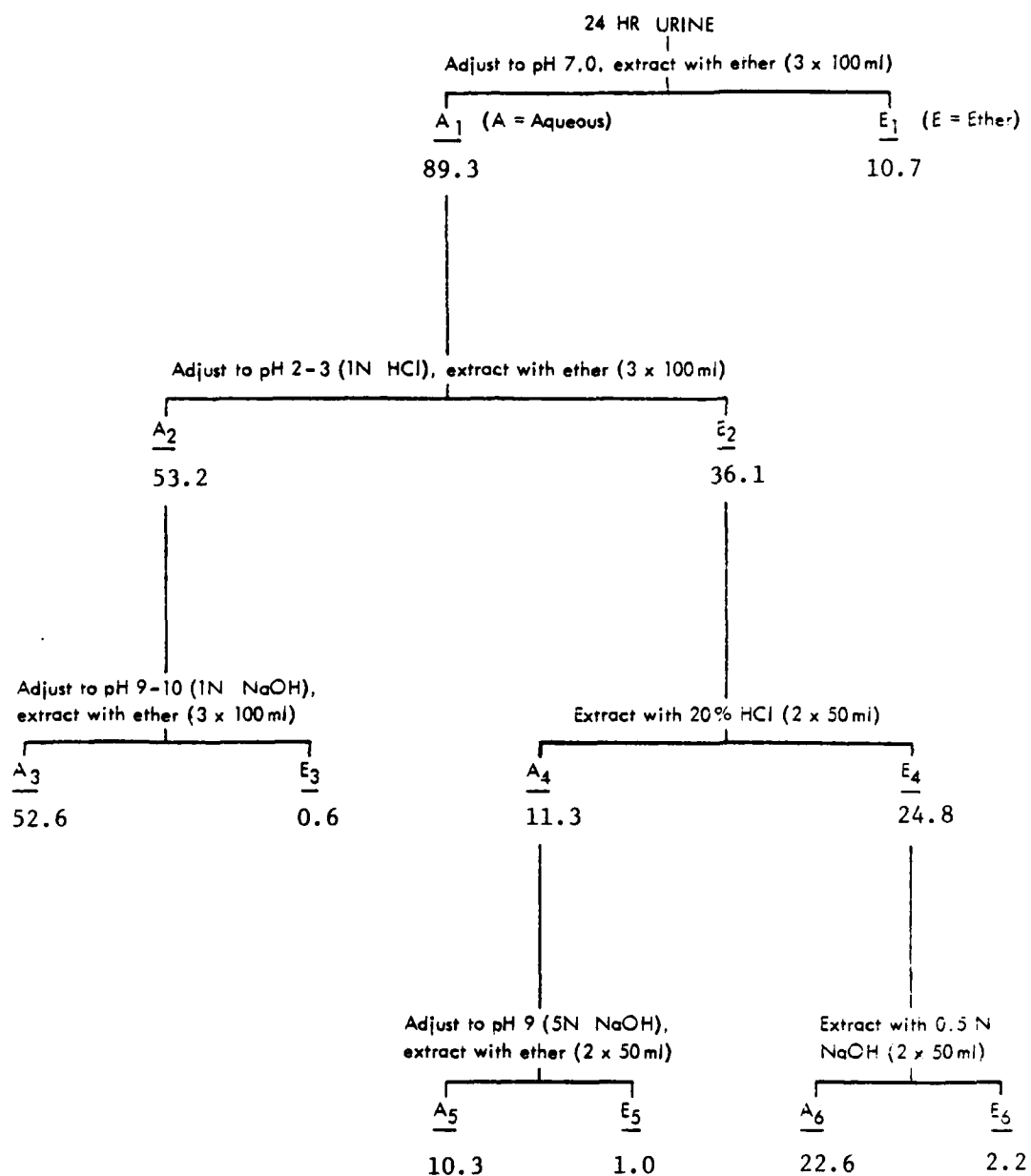


Figure 27: Fractionation of 24-Hr Urine Obtained from Rats Treated Dermally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 28, E₁-E₆: TLC of Ether Products Obtained from 24-Hr Urine of Rats Treated Dermal with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 28 follows

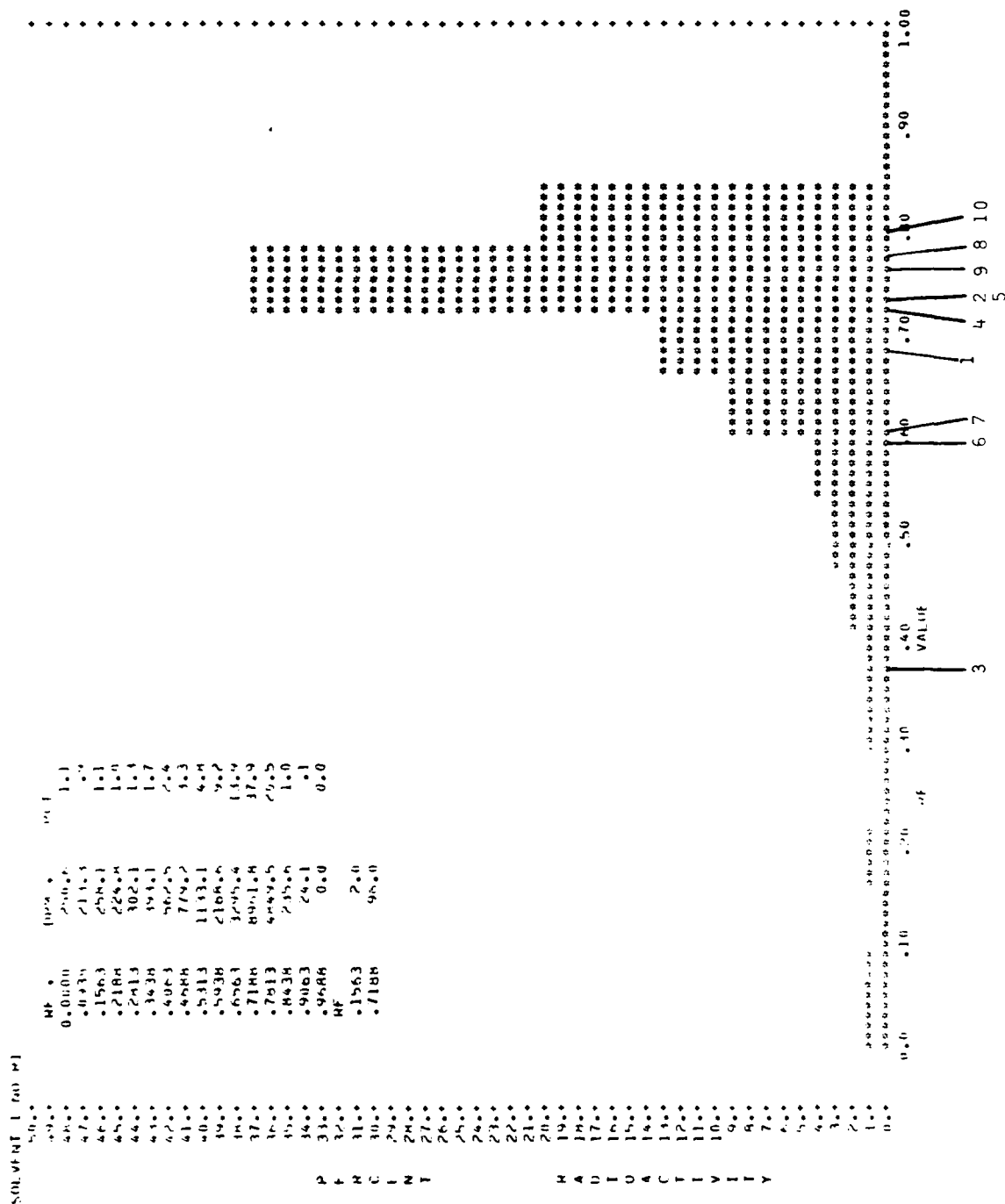


Figure 28-E1: Solvent I.



SOLVENT I NO 12

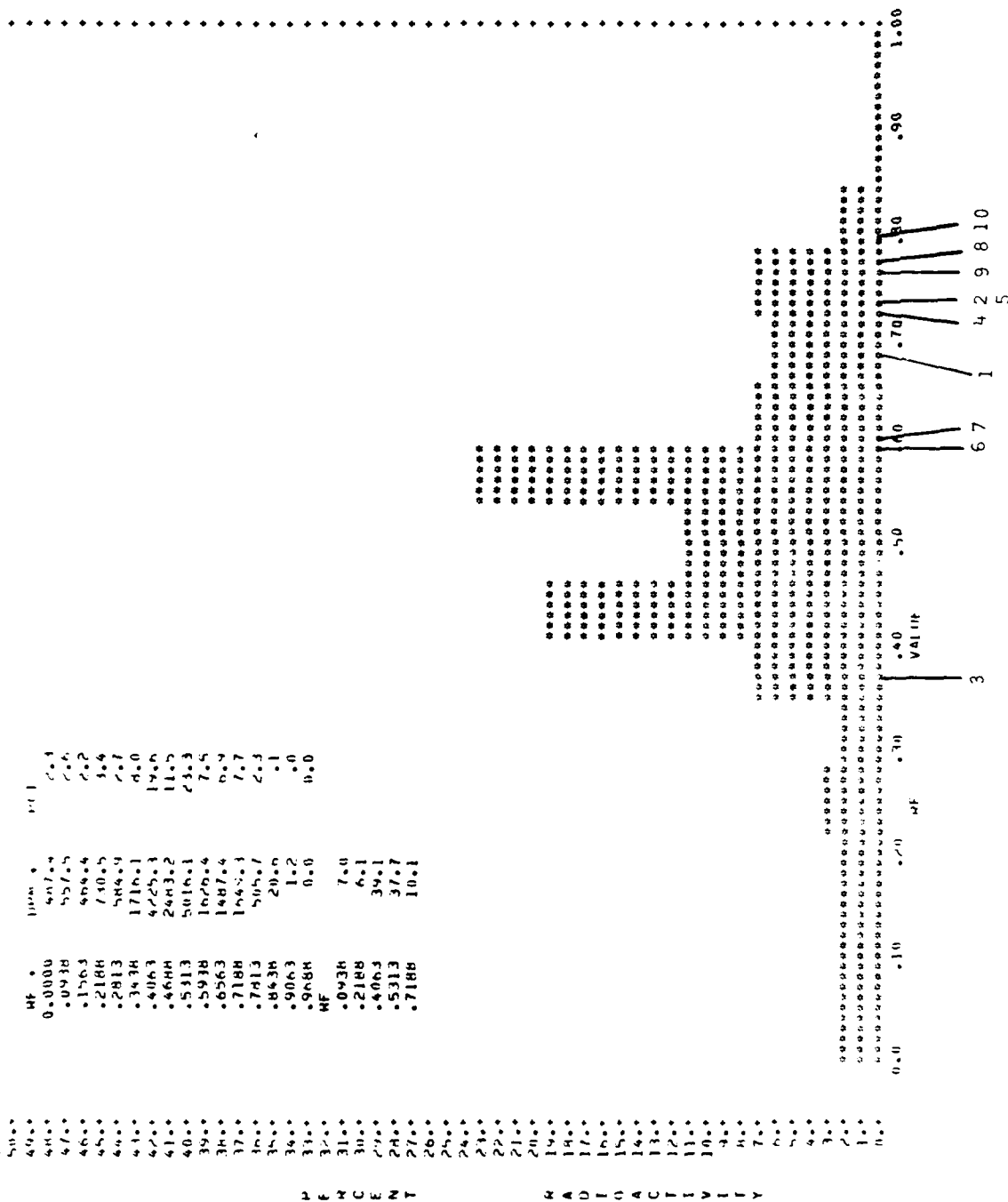
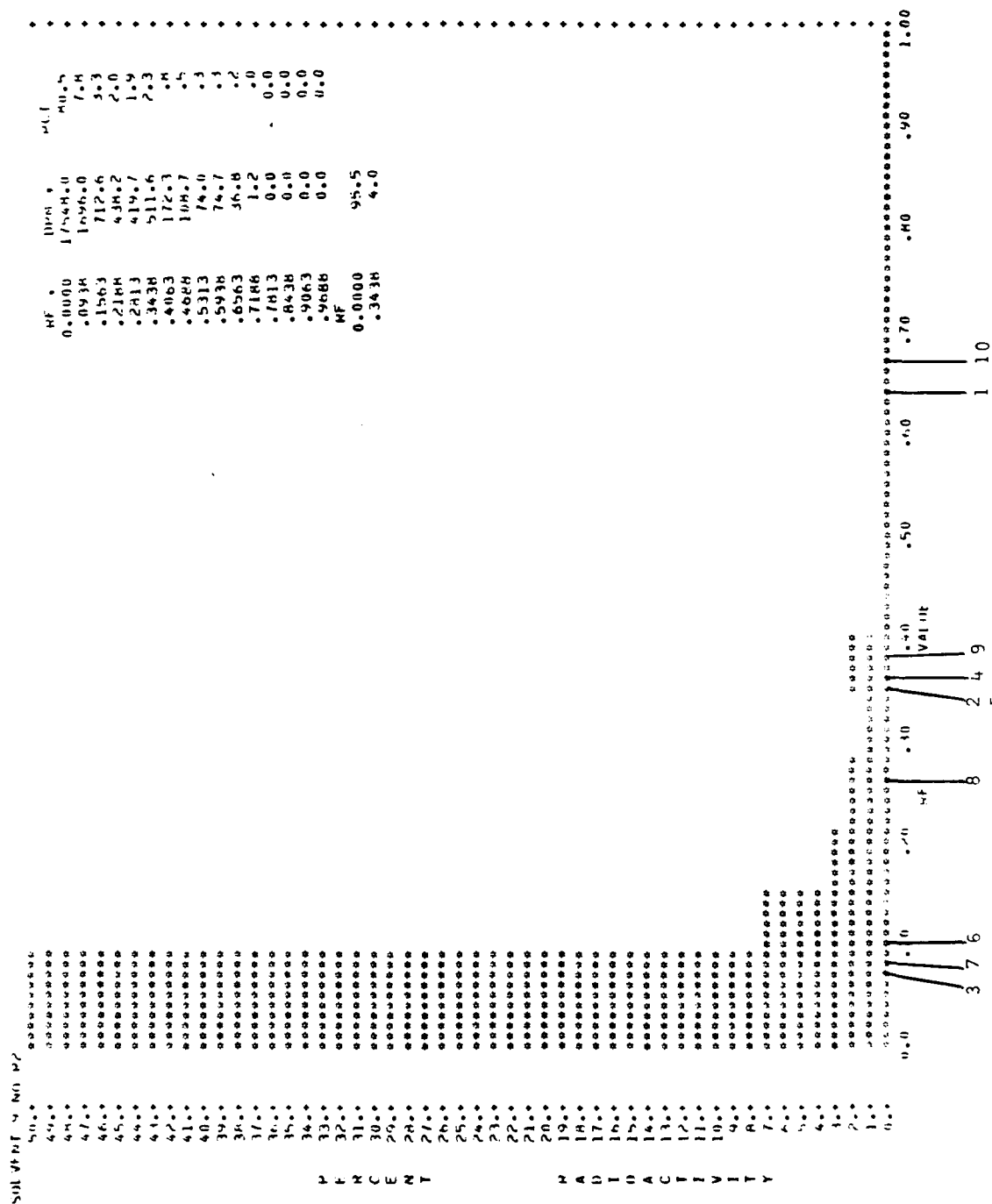


Figure 28-E2: Solvent I.



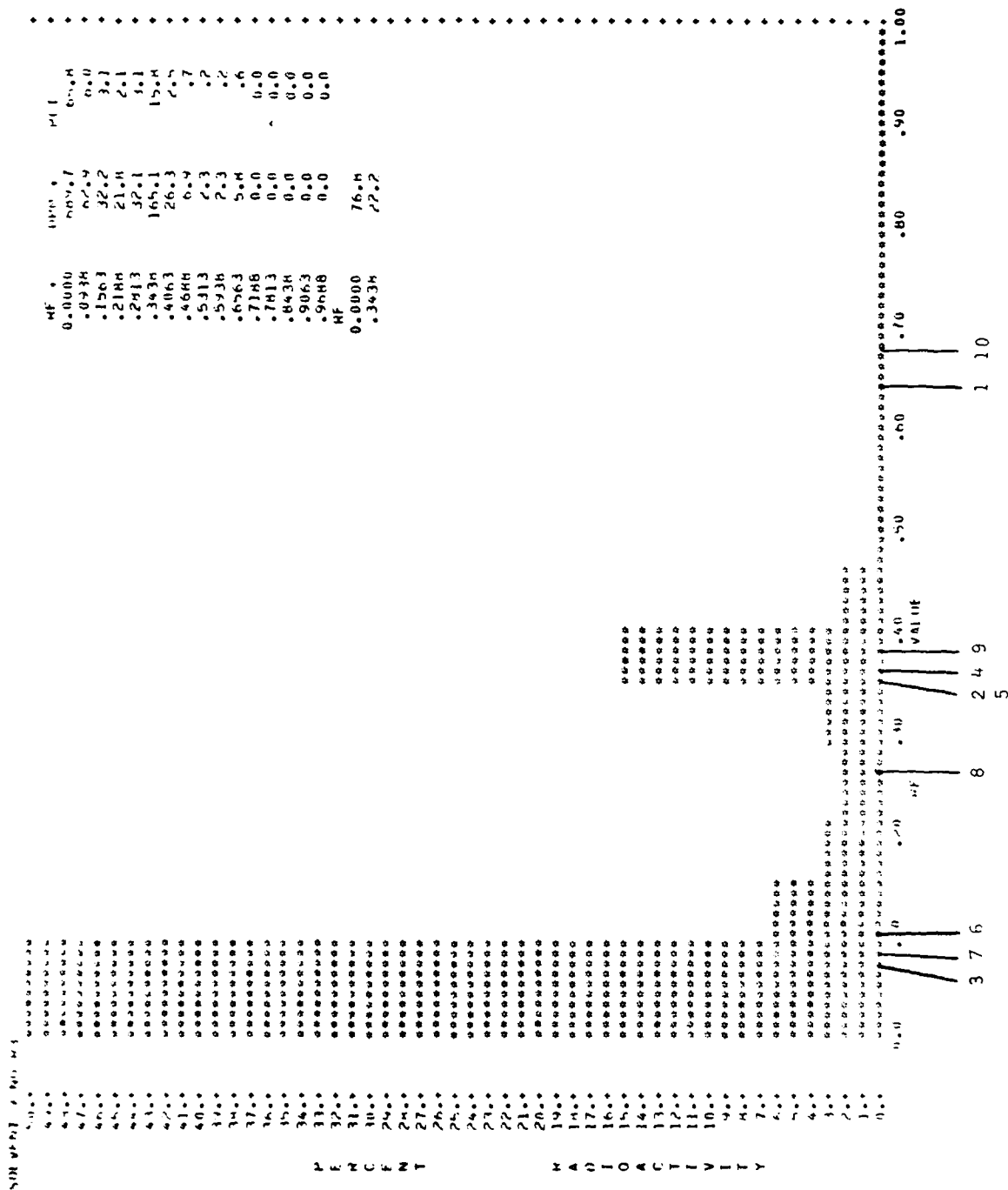


Figure 28-E₃: Solvent IX.

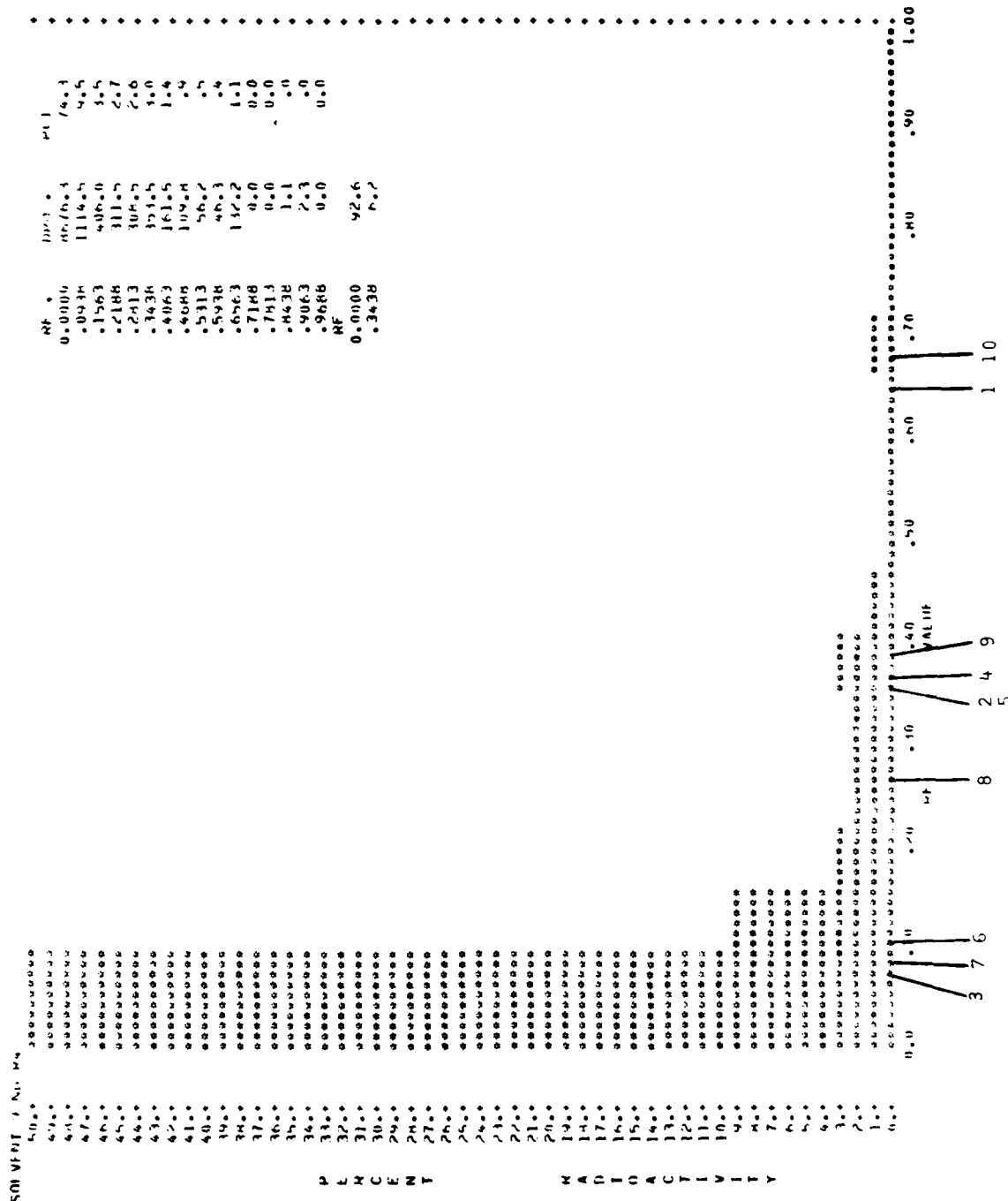
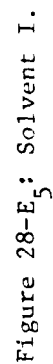


Figure 28-E₄: Solvent IX.



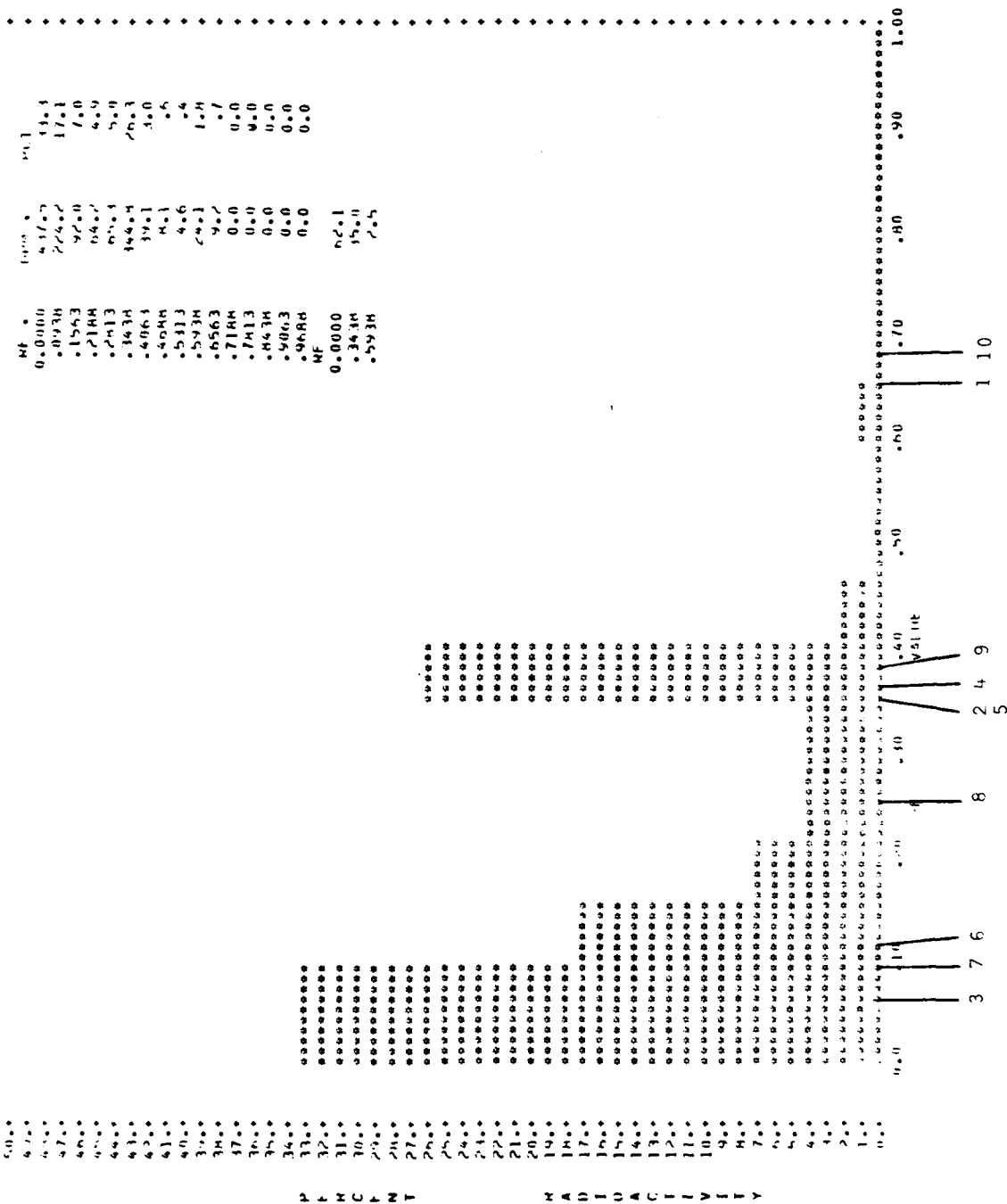
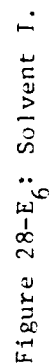


Figure 28-E₅: Solvent IX.



RF	1964	1965
0.000	34.54	14.54
0.038	12.54	7.54
1.563	154.54	6.54
2.186	162.54	1.54
2.413	142.54	6.54
3.338	682.3	24.7
4.063	128.4	7.0
4.668	74.7	4.4
5.113	63.6	2.8
5.478	117.9	5.1
6.563	265.2	12.5
7.184	4.6	5.2
7.413	0.0	0.0
8.438	0.0	0.0
9.063	1.2	1.1
9.688	7.3	1.1
RF	1966	1967
0.000	20.3	20.3
2.188	20.4	20.4
3.338	41.2	41.2
6.563	17.7	17.7

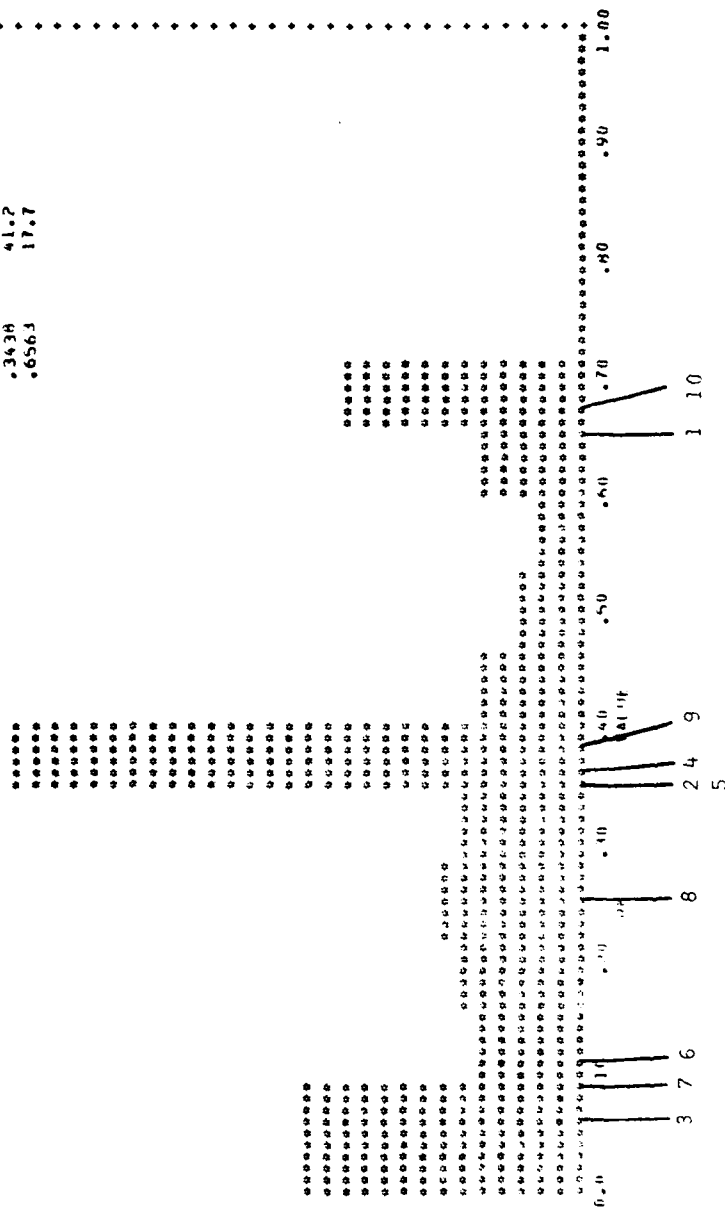


Figure 28-E₆: Solvent IX.

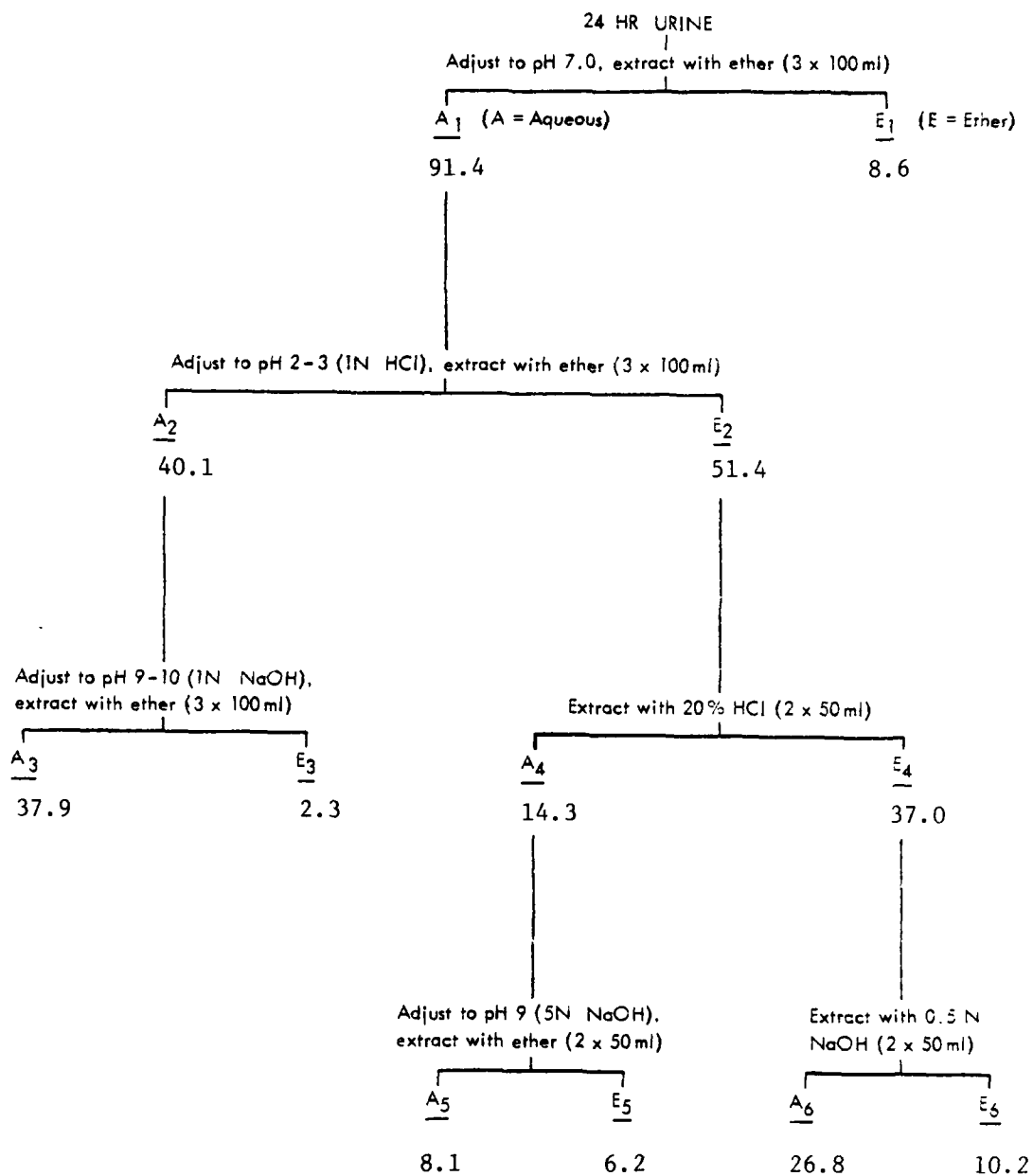


Figure 29: Fractionation of 24-Hr Urine Obtained from Mice Treated Orally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 30, E₁-E₆: TLC of Ether-Extractable Product Obtained from 24-Hr Urine of Mice Treated Orally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. For reference metabolites (1-10) see Figure 26 or Table 19. (E₅ fraction was spilled.) Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 30 follows

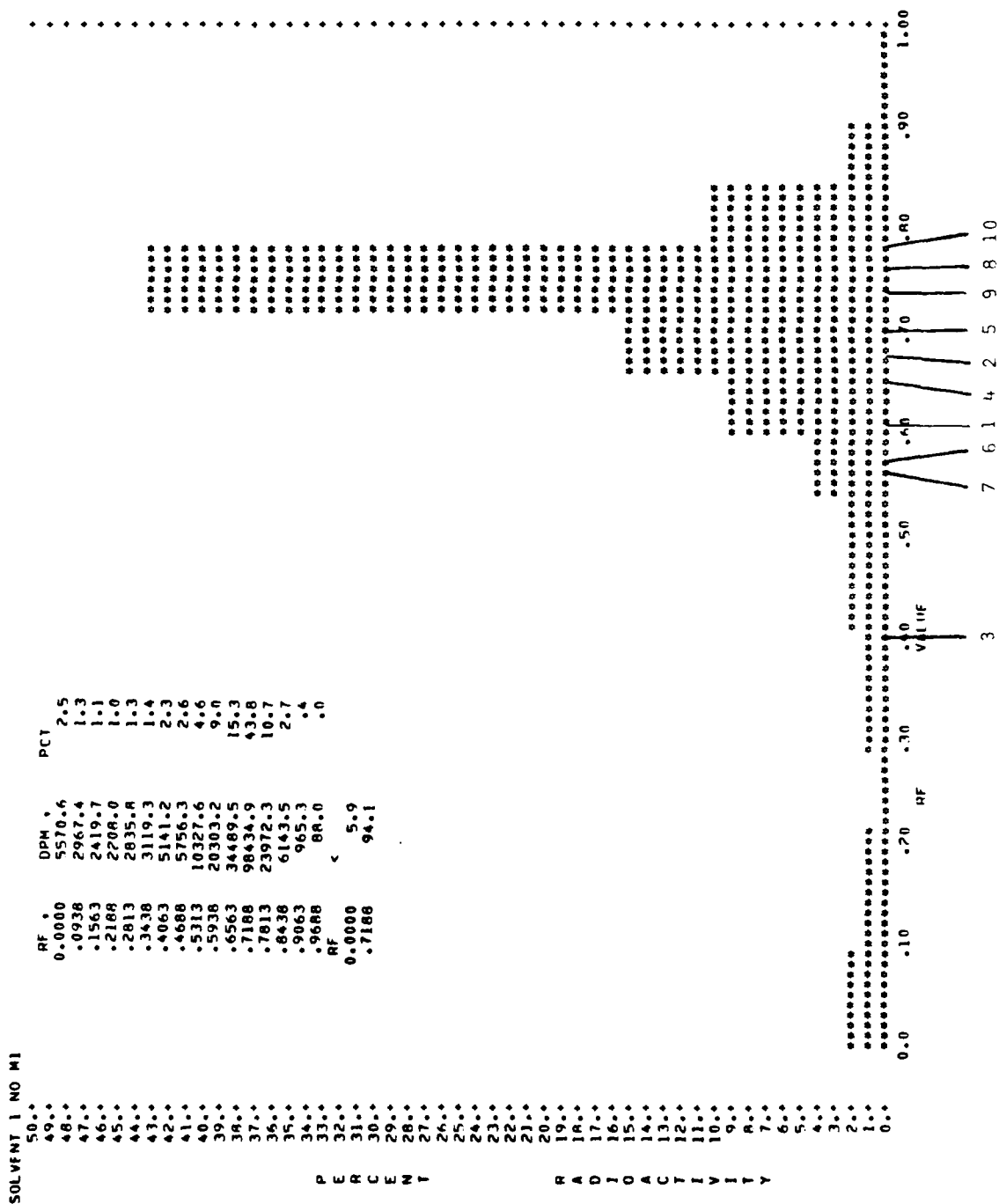


Figure 30-E₁: Solvent I

4274R JUNE 2R MICE AND DOG EXTRACTIONS SOLVENT 9 NO MI

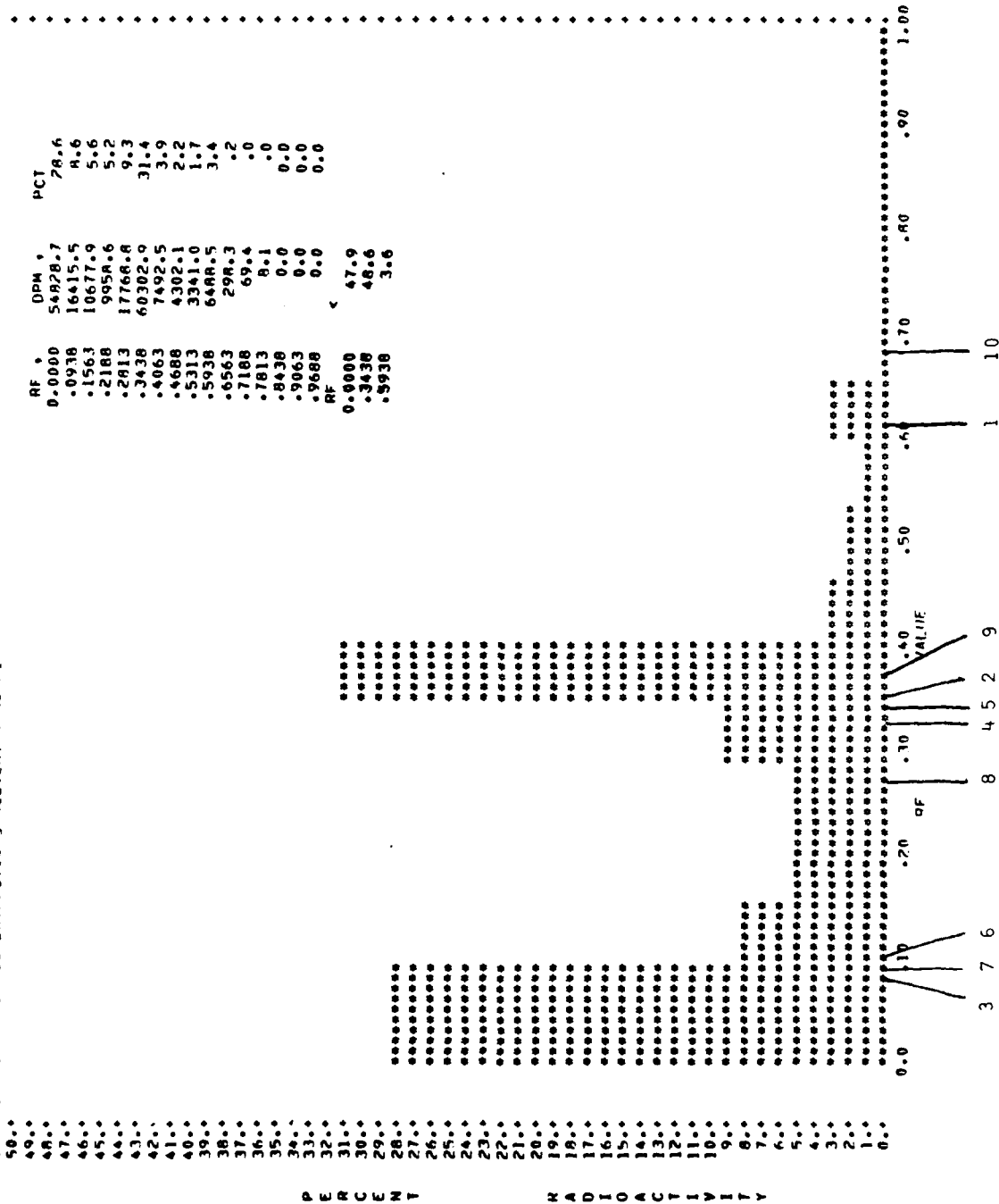


Figure 30-E1: Solvent IX

SOLVENT 1 NO M2

50.0	RF	DPM	PCT
49.0	0.0000	4936.4	3.7
48.0	.0938	3636.4	2.7
47.0	.1563	2931.8	2.2
46.0	.2188	4045.1	3.1
45.0	.2813	5670.1	4.3
44.0	.3438	9083.2	6.9
43.0	.4063	21966.5	16.6
42.0	.4688	15764.2	11.9
41.0	.5313	11259.0	8.5
40.0	.5938	11461.0	8.6
39.0	.6563	12848.4	9.7
38.0	.7188	24147.0	18.2
37.0	.7813	4355.8	3.3
36.0	.8438	341.0	.3
35.0	.9063	72.4	.1
34.0	.9688	9.3	.0
33.0	RF	<	
32.0	0.0000	8.7	
31.0	.4063	51.1	
30.0	.7188	40.2	

P E R C C E N T

R A D I O A C T I V I T Y

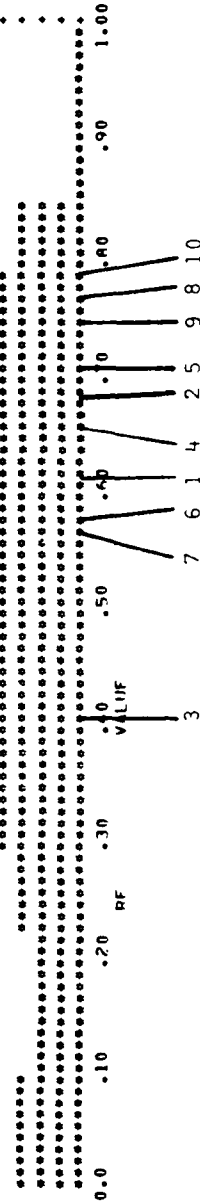


Figure 30-E2: Solvent I

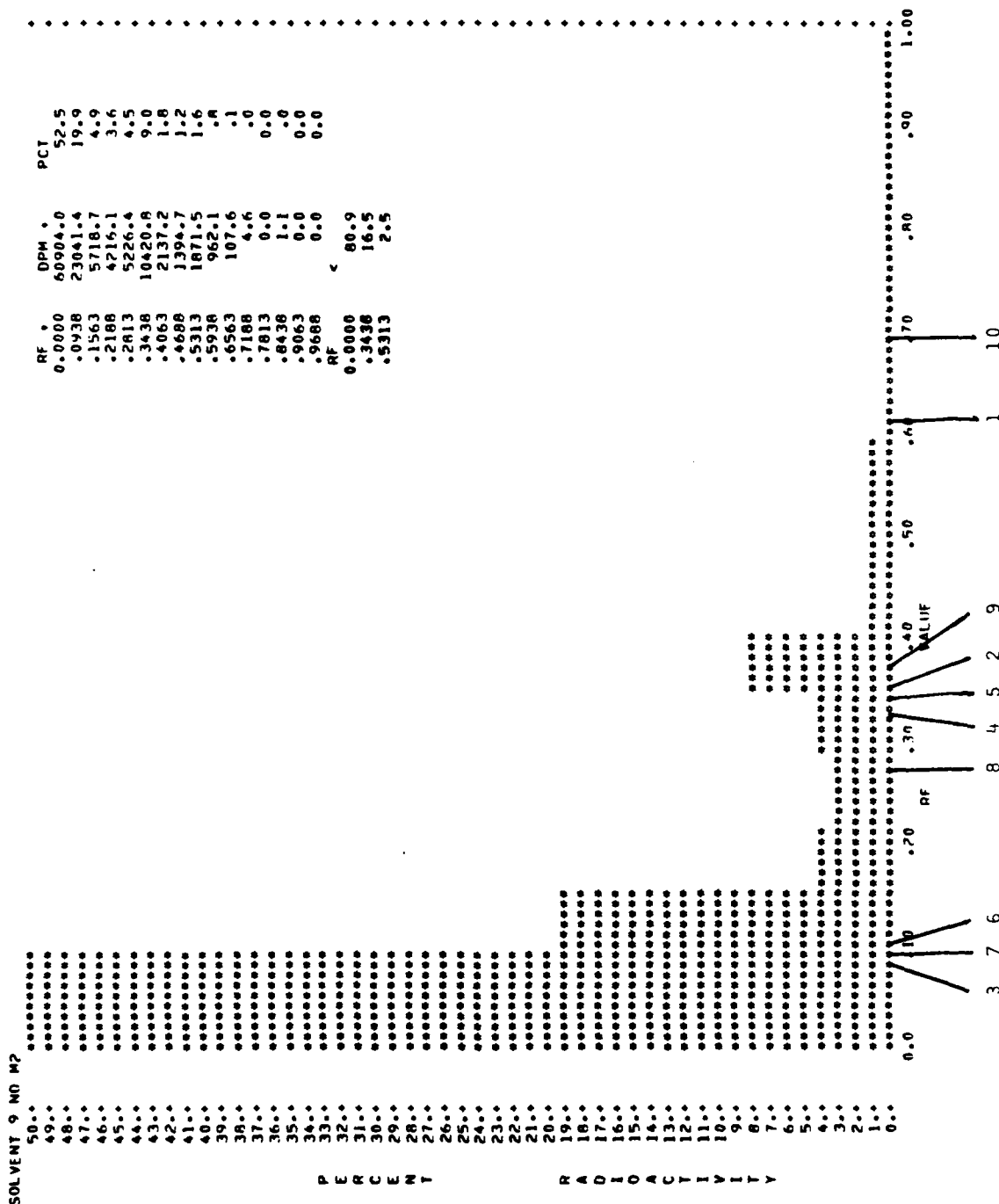


Figure 30-E2: Solvent IX

SOLVENT 1 NO M3

RF	DPM	PCT
0.0000	1604.6	3.8
.0938	614.9	1.5
.1563	594.9	1.4
.2108	538.7	1.3
.2813	553.3	1.3
.3438	659.8	1.6
.4063	840.5	2.0
.4688	1079.9	2.5
.5313	1517.4	3.6
.5938	2077.5	4.9
.6563	5276.7	12.5
.7188	23667.8	55.9
.7813	2983.9	7.0
.8438	297.5	.7
.9063	47.1	.1
.9688	12.7	.0
RF	<	
0.0000	7.9	
.7188	92.1	

P E R C E N T

R A D I O A C T I V I T Y

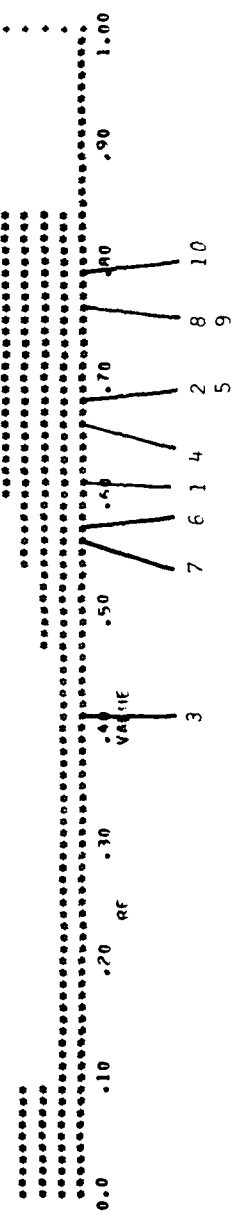


Figure 30-E₃: Solvent I

SOLVENT 9 NO 43

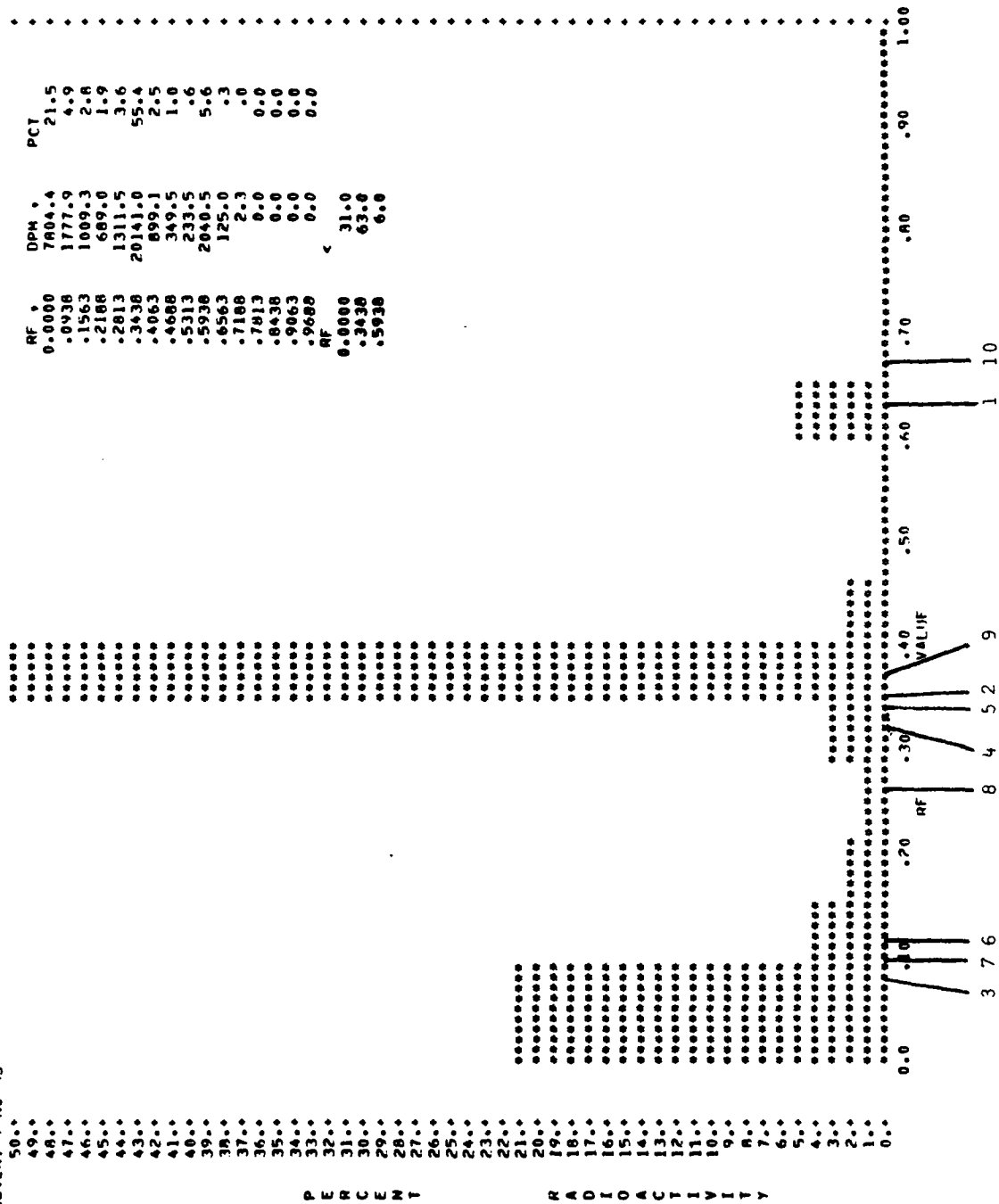


Figure 30-E3: Solvent IX

SOLVENT 1 NO M4

50.0	RF	DPM	PCT
49.0	0.0000	3506.4	3.8
48.0	.0938	2010.4	2.2
47.0	.1563	2064.7	2.2
46.0	.2188	2047.1	4.1
45.0	.2813	3856.5	3.5
44.0	.3438	3263.9	17.1
43.0	.4063	15986.1	9.6
42.0	.4688	8963.0	6.6
41.0	.5313	6183.1	7.8
40.0	.5938	7284.0	10.5
39.0	.6563	9809.2	22.1
38.0	.7188	20653.2	7.4
37.0	.7813	6926.0	.7
36.0	.8438	664.4	.2
35.0	.9063	143.2	.0
34.0	.9688	24.3	
33.0	RF	<	
32.0	0.0000	5.9	
31.0	.1563	4.4	
30.0	.2813	7.6	
29.0	.4063	33.3	
28.0	.7188	48.7	

P E R C E N T

H A D I O A C T I V I T Y

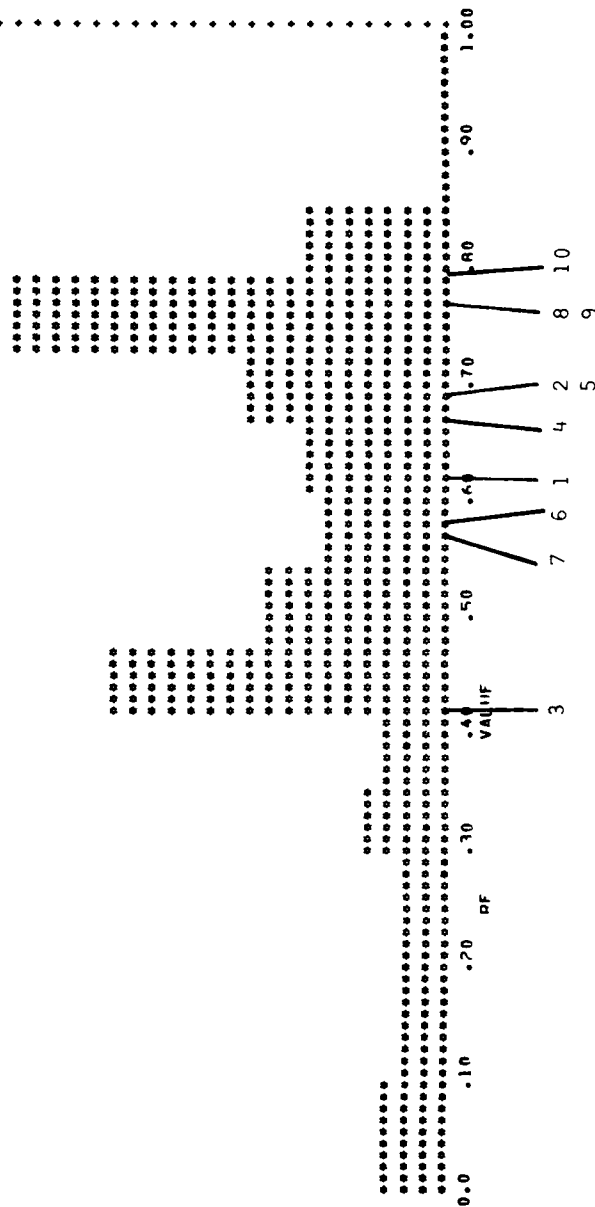


Figure 30-E4: Solvent I

SOLVENT 9 NO M4

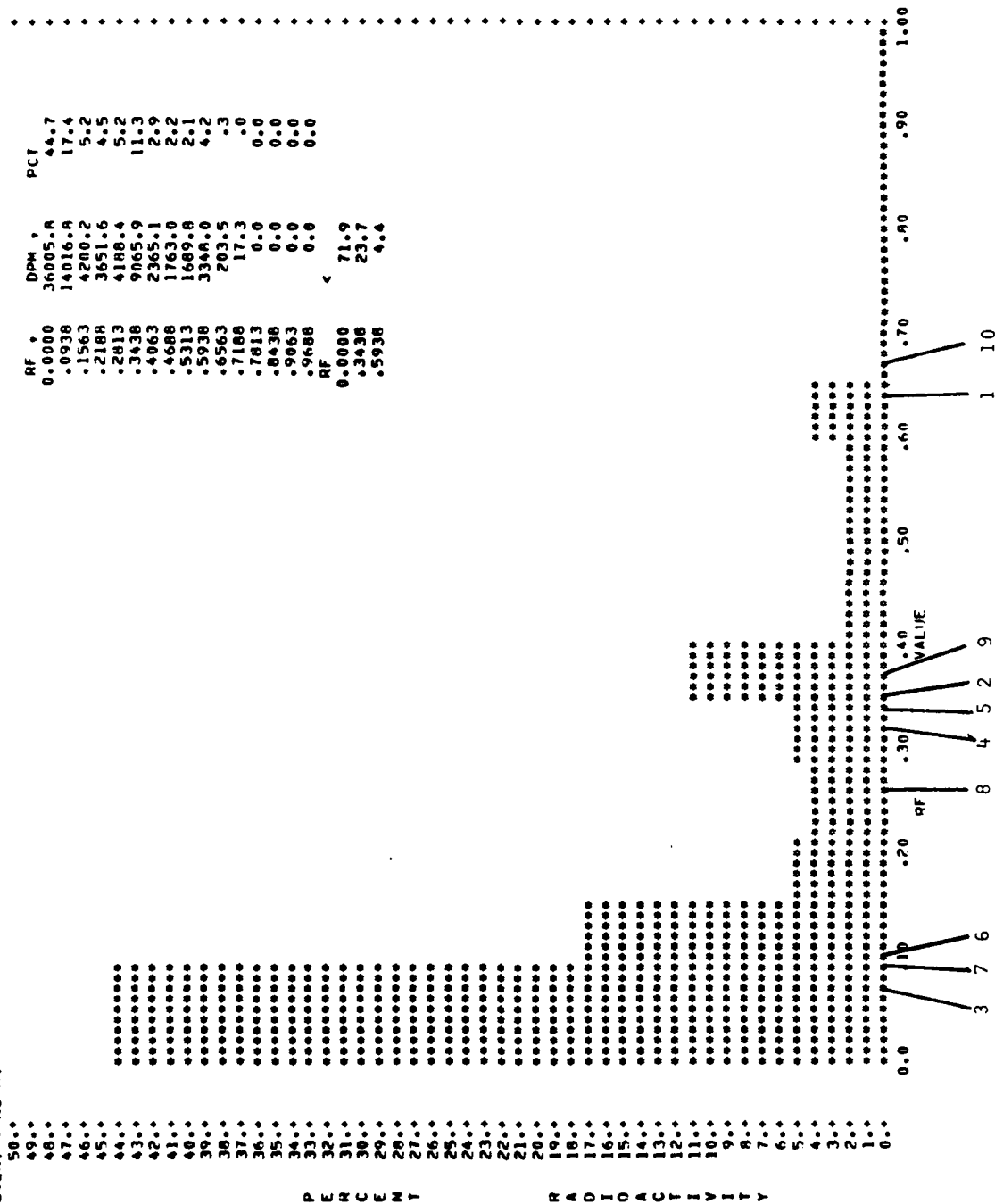


Figure 30-E4: Solvent IX

SOLVENT 1 NO M4

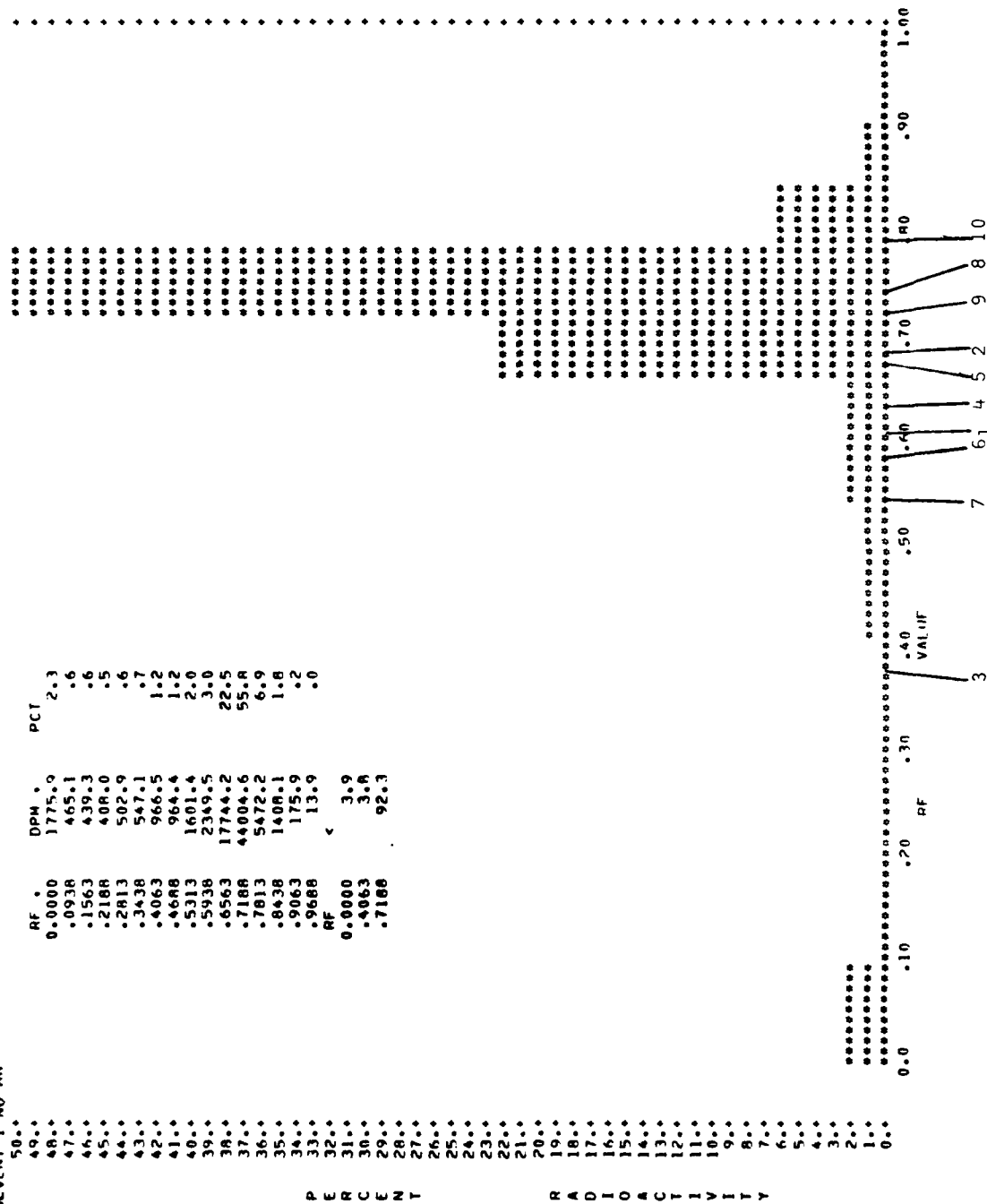


Figure 30-E6: Solvent I

SOLVENT 9 NO M6

50.0	RF	0.0000	DPH	4778.0	PCT	6.8
49.0		.0938		1732.9		2.5
48.0		.1563		1984.9		2.8
47.0		.2188		2454.7		3.5
46.0		.2813		2857.8		4.1
45.0		.3438		2986.0		42.1
44.0		.4063		3388.9		4.8
43.0		.4688		2187.6		3.1
42.0		.5313		3251.7		4.6
41.0		.5938		16323.7		23.3
40.0		.6563		1538.7		2.2
39.0		.7188		65.9		.1
38.0		.7813		0.0		0.0
37.0		.8438		0.0		0.0
36.0		.9063		0.0		0.0
35.0		.9688		0.0		0.0
34.0						
33.0						
32.0						
31.0						
30.0						
29.0						
28.0						
27.0						
26.0						
25.0						
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22.0						
21.0						
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19.0						
18.0						
17.0						
16.0						
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14.0						
13.0						
12.0						
11.0						
10.0						
9.0						
8.0						
7.0						
6.0						
5.0						
4.0						
3.0						
2.0						
1.0						
0.0						

P E R C E N T

R A D I O A C T I V I T Y

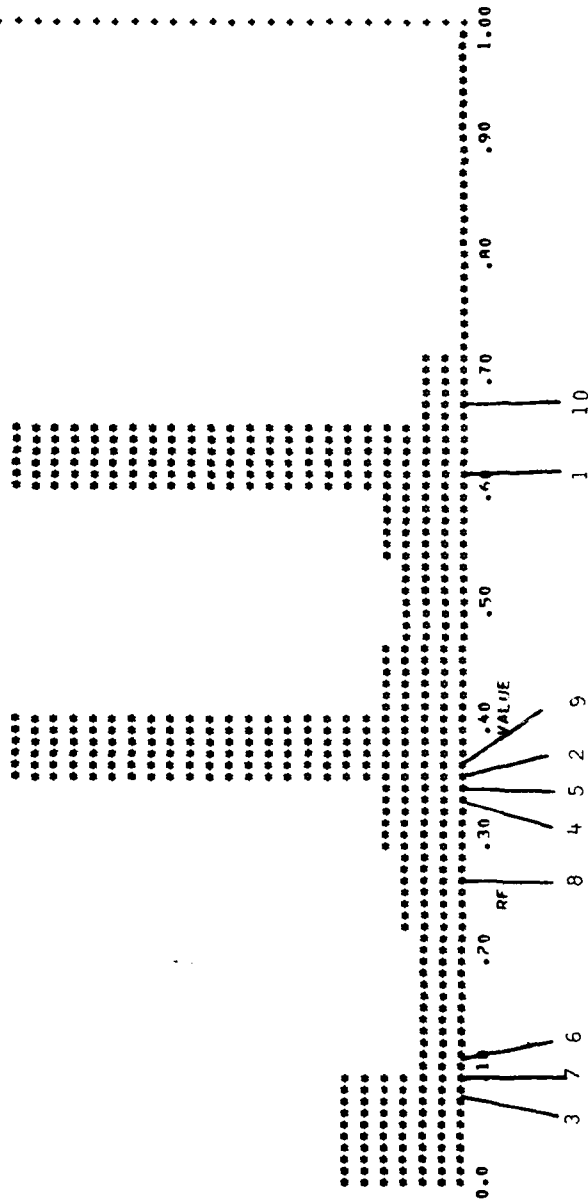


Figure 30-E6: Solvent IX

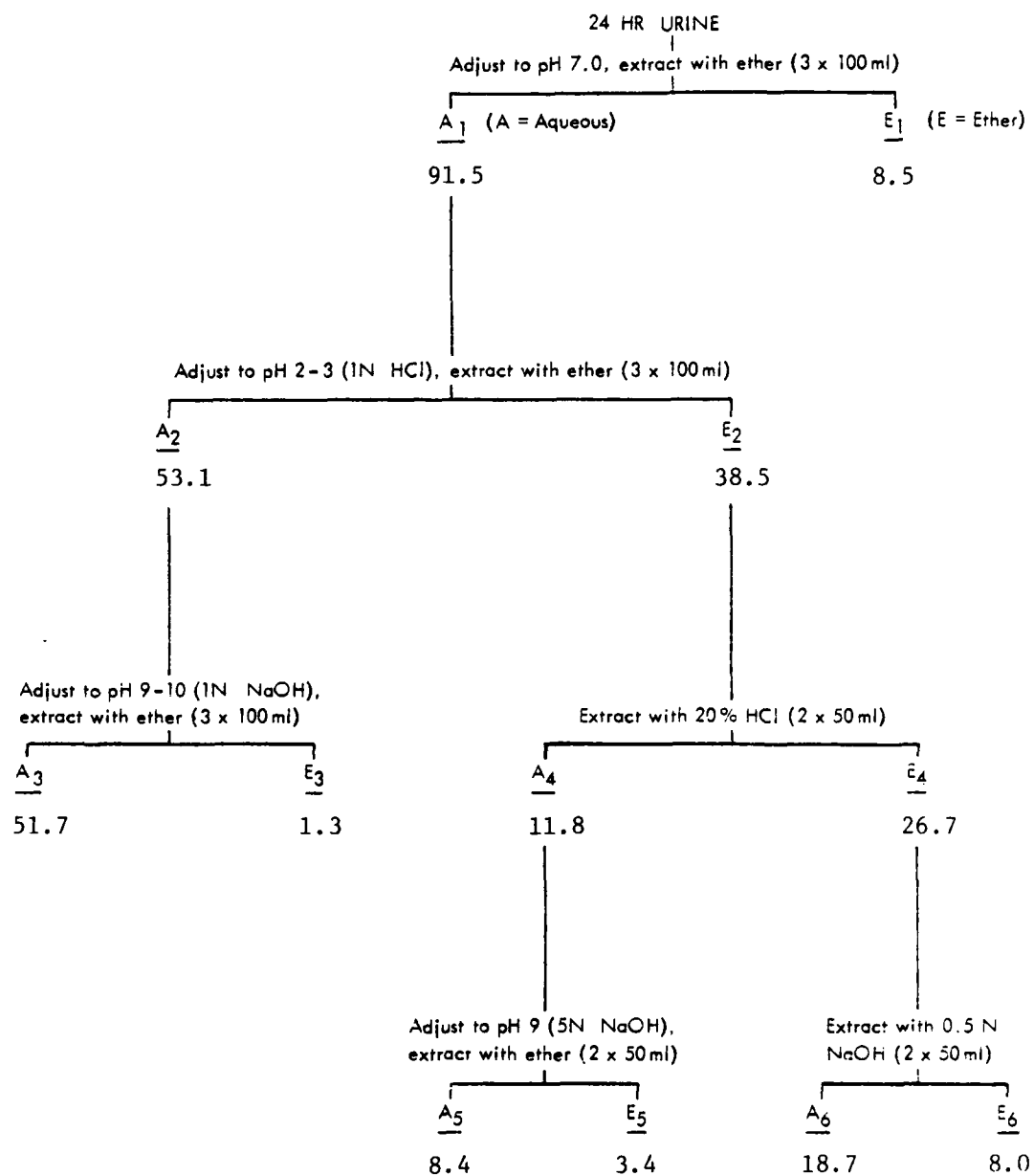


Figure 31: Fractionation of 24-Hr Urine Obtained from Mice Treated Dermally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 32: TLC of Ether-Extractable Products Obtained from 24-Hr Urine of Mice Treated Dermal with ^{14}C -TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 10L1:1; Solvent IX, toluene:acetic acid, 4:1. For reference metabolites (1-10) see Figure 26 or Table 19. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 32 follows

SOLVENT 1 NO. 41

50.0	40.0	30.0	20.0	10.0	0.0
43.0	42.0	41.0	40.0	39.0	38.0
47.0	46.0	45.0	44.0	43.0	42.0
46.0	45.0	44.0	43.0	42.0	41.0
49.0	48.0	47.0	46.0	45.0	44.0
44.0	43.0	42.0	41.0	40.0	39.0
42.0	41.0	40.0	39.0	38.0	37.0
41.0	40.0	39.0	38.0	37.0	36.0
40.0	39.0	38.0	37.0	36.0	35.0
39.0	38.0	37.0	36.0	35.0	34.0
38.0	37.0	36.0	35.0	34.0	33.0
37.0	36.0	35.0	34.0	33.0	32.0
36.0	35.0	34.0	33.0	32.0	31.0
35.0	34.0	33.0	32.0	31.0	30.0
34.0	33.0	32.0	31.0	30.0	29.0
33.0	32.0	31.0	30.0	29.0	28.0
32.0	31.0	30.0	29.0	28.0	27.0
31.0	30.0	29.0	28.0	27.0	26.0
30.0	29.0	28.0	27.0	26.0	25.0
29.0	28.0	27.0	26.0	25.0	24.0
28.0	27.0	26.0	25.0	24.0	23.0
27.0	26.0	25.0	24.0	23.0	22.0
26.0	25.0	24.0	23.0	22.0	21.0
25.0	24.0	23.0	22.0	21.0	20.0
24.0	23.0	22.0	21.0	20.0	19.0
23.0	22.0	21.0	20.0	19.0	18.0
22.0	21.0	20.0	19.0	18.0	17.0
21.0	20.0	19.0	18.0	17.0	16.0
20.0	19.0	18.0	17.0	16.0	15.0
19.0	18.0	17.0	16.0	15.0	14.0
18.0	17.0	16.0	15.0	14.0	13.0
17.0	16.0	15.0	14.0	13.0	12.0
16.0	15.0	14.0	13.0	12.0	11.0
15.0	14.0	13.0	12.0	11.0	10.0
14.0	13.0	12.0	11.0	10.0	9.0
13.0	12.0	11.0	10.0	9.0	8.0
12.0	11.0	10.0	9.0	8.0	7.0
11.0	10.0	9.0	8.0	7.0	6.0
10.0	9.0	8.0	7.0	6.0	5.0
9.0	8.0	7.0	6.0	5.0	4.0
8.0	7.0	6.0	5.0	4.0	3.0
7.0	6.0	5.0	4.0	3.0	2.0
6.0	5.0	4.0	3.0	2.0	1.0
5.0	4.0	3.0	2.0	1.0	0.0
4.0	3.0	2.0	1.0	0.0	
3.0	2.0	1.0	0.0		
2.0	1.0	0.0			
1.0	0.0				
0.0					

P F M C E N T

R A D I C I C I V I T Y

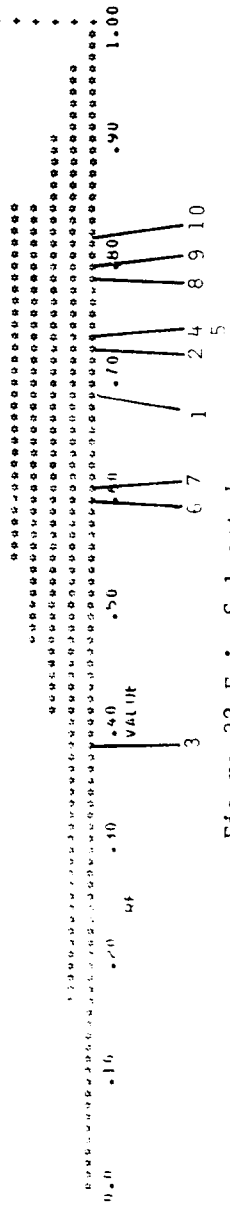


Figure 32-E1: Solvent 1

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MIDWEST RESEARCH INST KANSAS CITY MO
SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM OF 2,4,6---ETC(U)
JUN 81 A M EL-HAWARI, J R HODGSON

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27th JULY 28 1976 MICE AND RAT EXTENSIVE SOLVENT 9 40 MJ

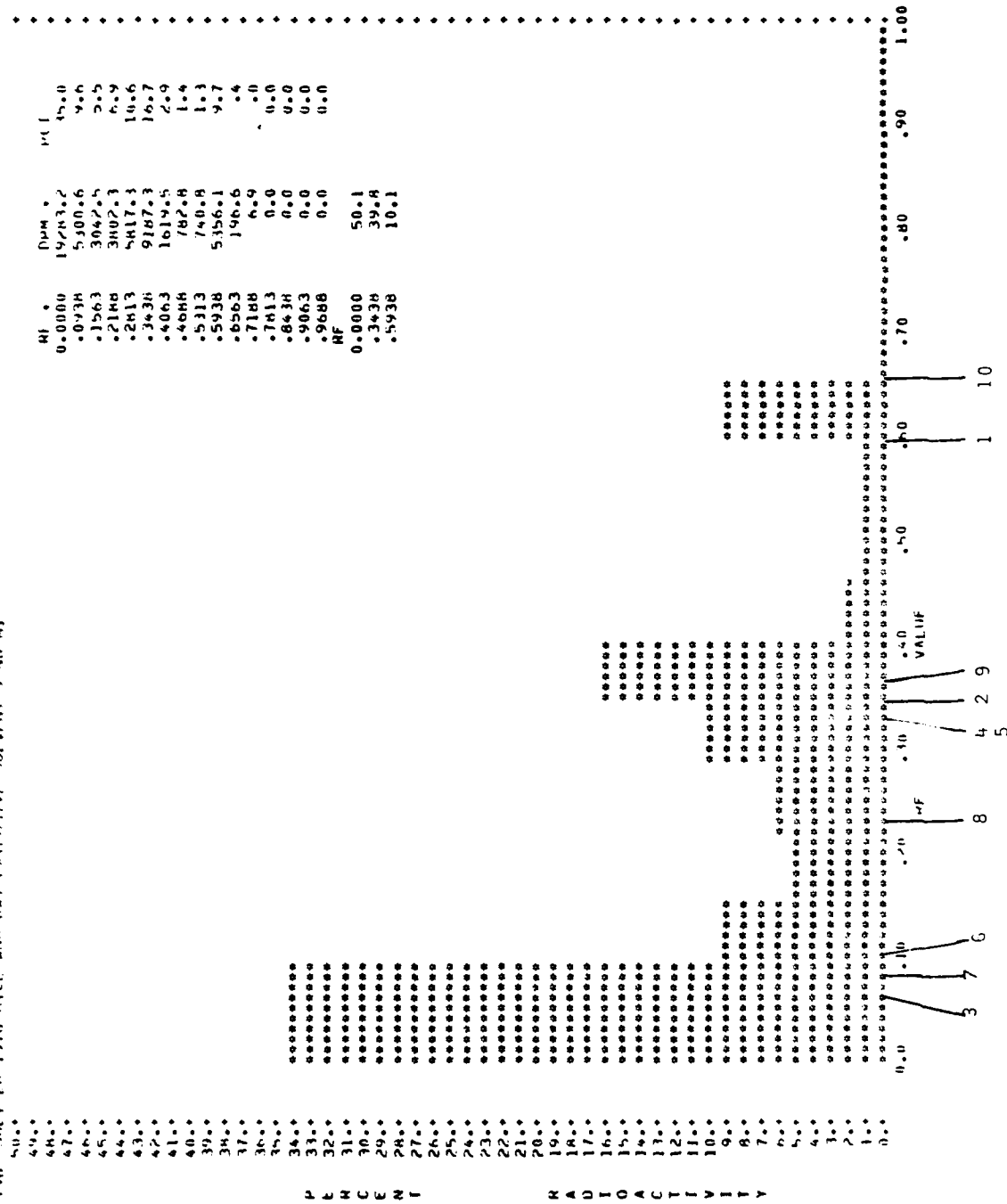


Figure 32-E1: Solvent IX

SOLVENT I 100 wt

50.0	100.0	100.0	100.0
47.0	97.0	97.0	97.0
46.0	96.0	96.0	96.0
45.0	95.0	95.0	95.0
44.0	94.0	94.0	94.0
43.0	93.0	93.0	93.0
42.0	92.0	92.0	92.0
41.0	91.0	91.0	91.0
40.0	90.0	90.0	90.0
39.0	89.0	89.0	89.0
38.0	88.0	88.0	88.0
37.0	87.0	87.0	87.0
36.0	86.0	86.0	86.0
35.0	85.0	85.0	85.0
34.0	84.0	84.0	84.0
33.0	83.0	83.0	83.0
32.0	82.0	82.0	82.0
31.0	81.0	81.0	81.0
30.0	80.0	80.0	80.0
29.0	79.0	79.0	79.0
28.0	78.0	78.0	78.0
27.0	77.0	77.0	77.0
26.0	76.0	76.0	76.0
25.0	75.0	75.0	75.0
24.0	74.0	74.0	74.0
23.0	73.0	73.0	73.0
22.0	72.0	72.0	72.0
21.0	71.0	71.0	71.0
20.0	70.0	70.0	70.0
19.0	69.0	69.0	69.0
18.0	68.0	68.0	68.0
17.0	67.0	67.0	67.0
16.0	66.0	66.0	66.0
15.0	65.0	65.0	65.0
14.0	64.0	64.0	64.0
13.0	63.0	63.0	63.0
12.0	62.0	62.0	62.0
11.0	61.0	61.0	61.0
10.0	60.0	60.0	60.0
9.0	59.0	59.0	59.0
8.0	58.0	58.0	58.0
7.0	57.0	57.0	57.0
6.0	56.0	56.0	56.0
5.0	55.0	55.0	55.0
4.0	54.0	54.0	54.0
3.0	53.0	53.0	53.0
2.0	52.0	52.0	52.0
1.0	51.0	51.0	51.0
0.0	50.0	50.0	50.0

P F R C E N T

H A D I O A C I I V I F Y

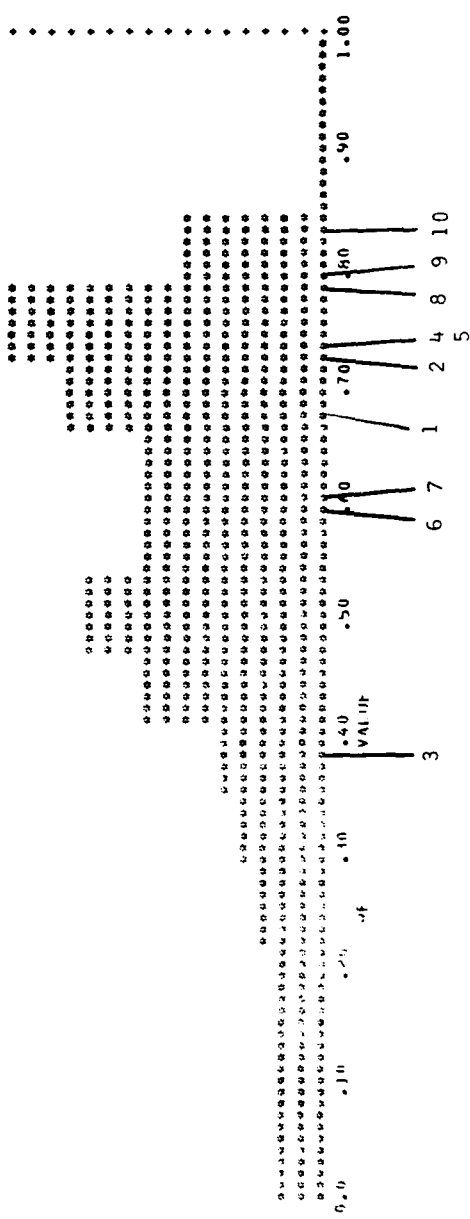


Figure 32-E₂: Solvent I

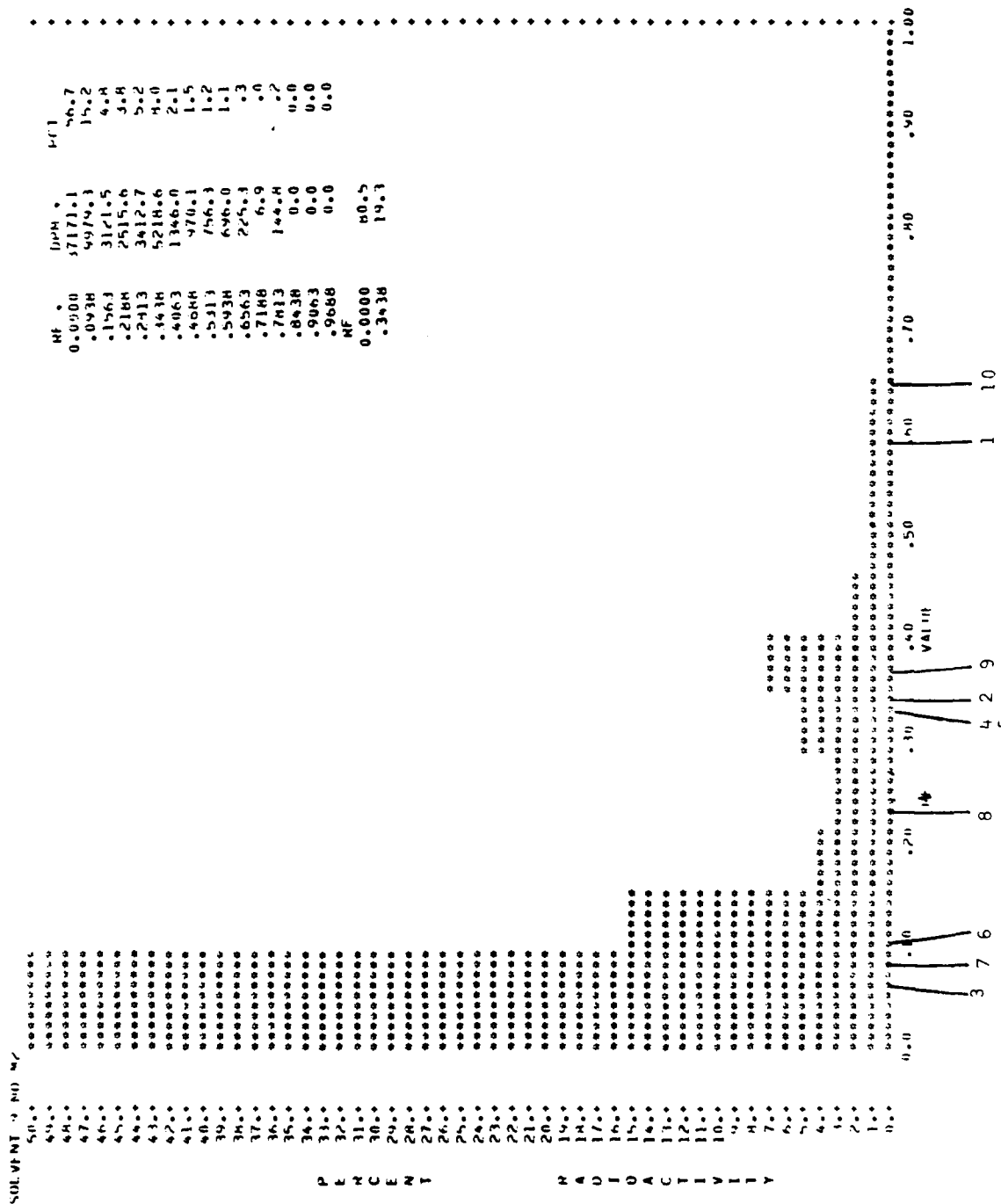


Figure 32-E₂: Solvent IX

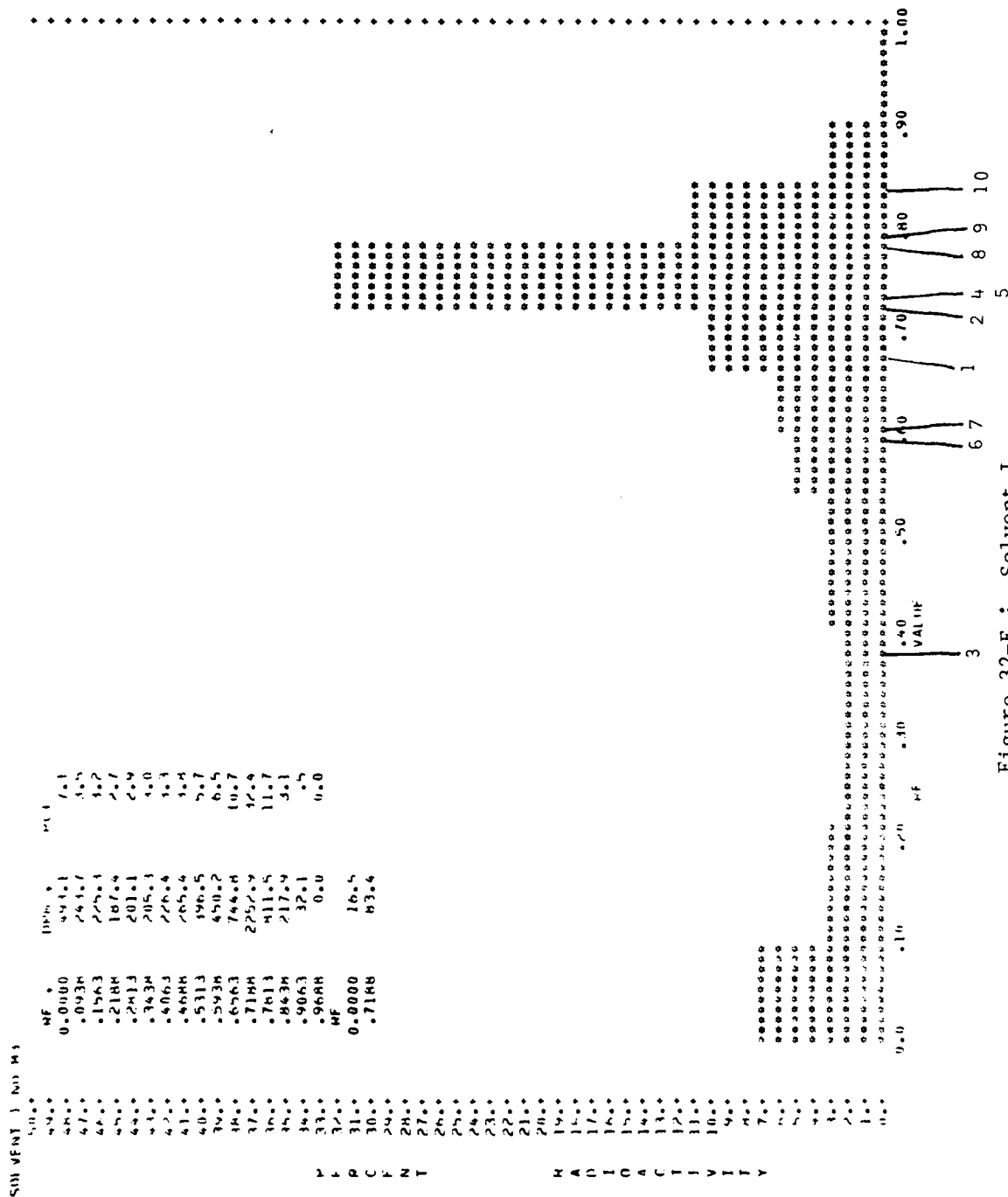
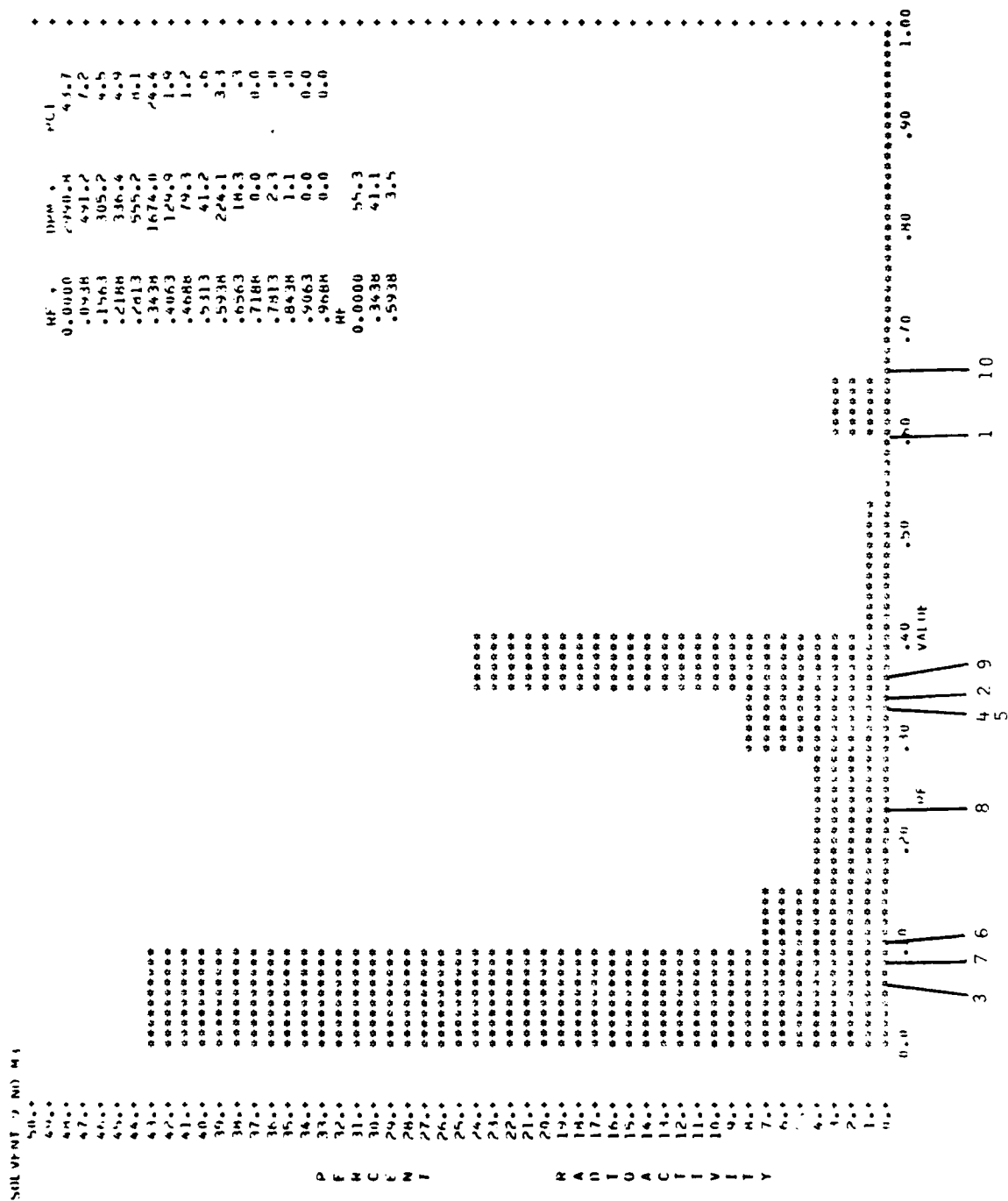


Figure 32-E₃: Solvent I



SUB UNIT 1 NO. 2

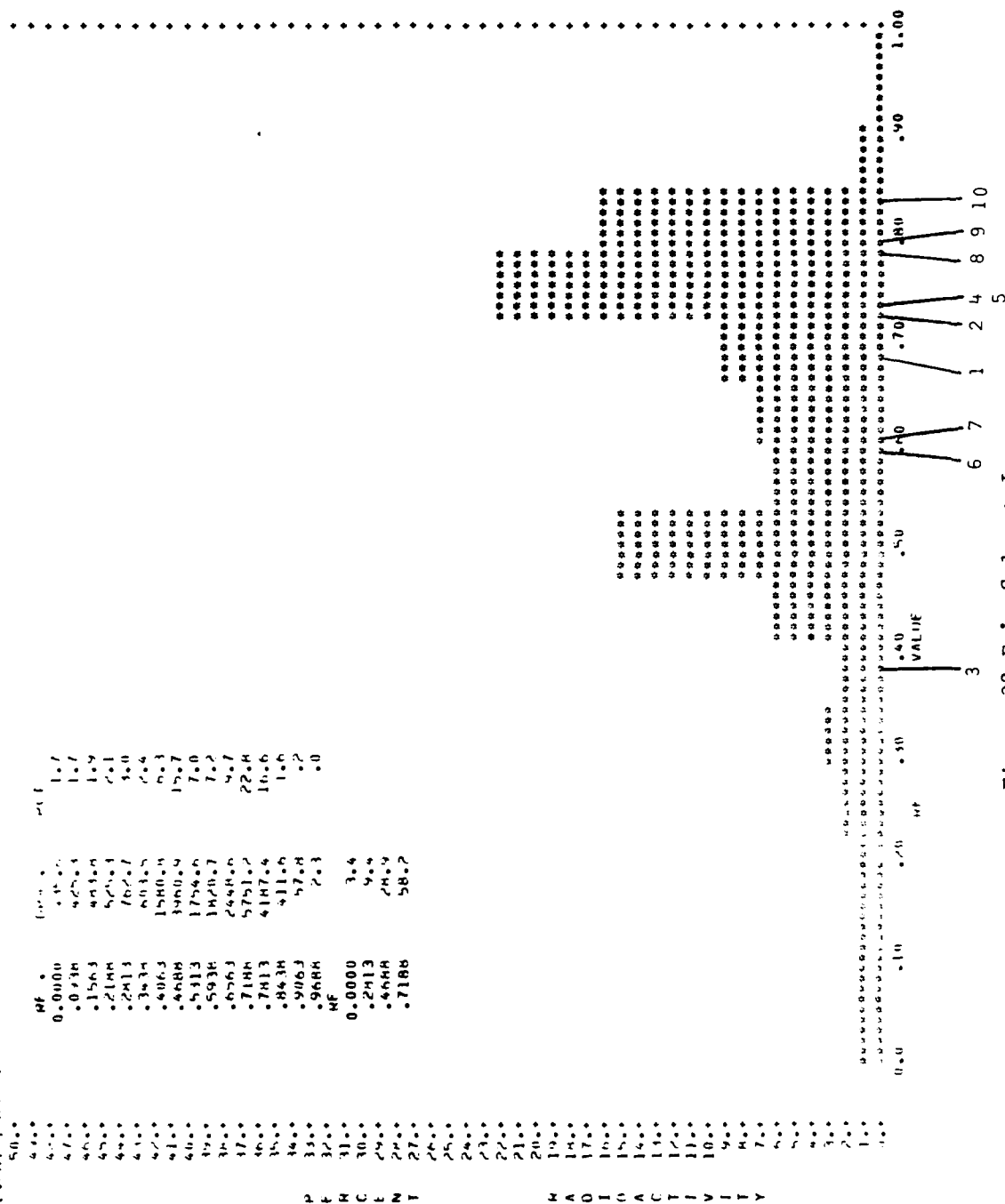


Figure 32-E₄: Solvent I

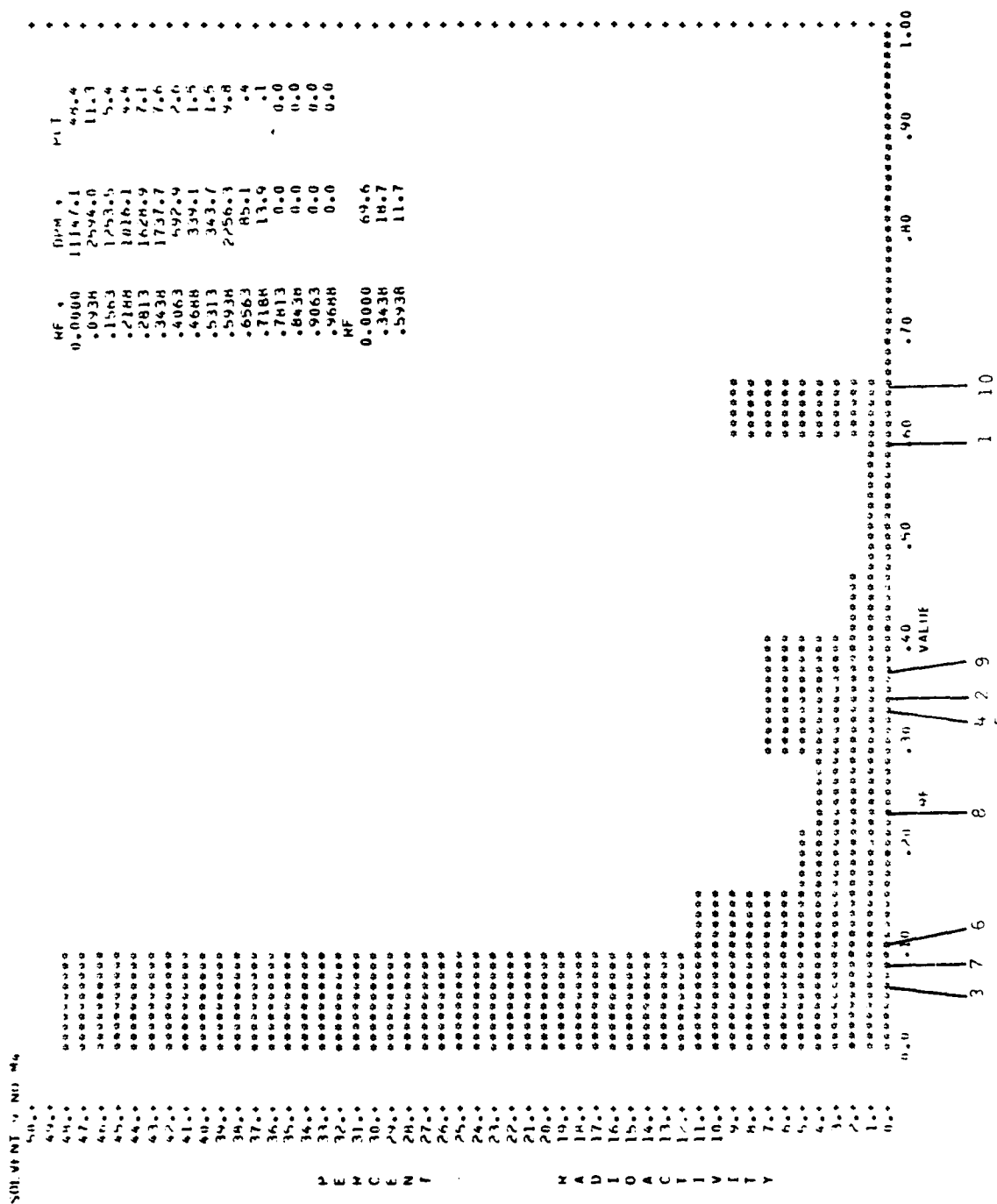
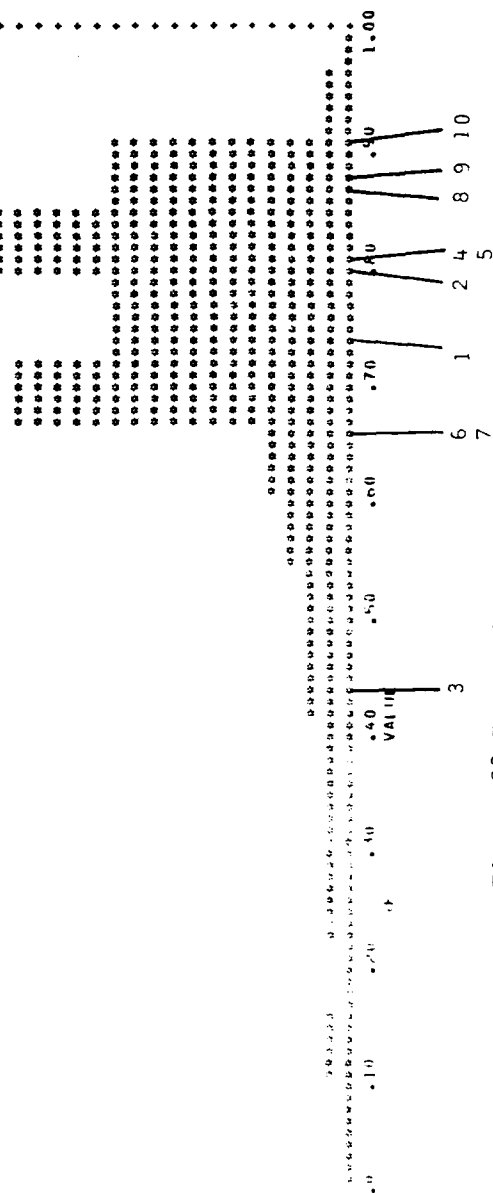


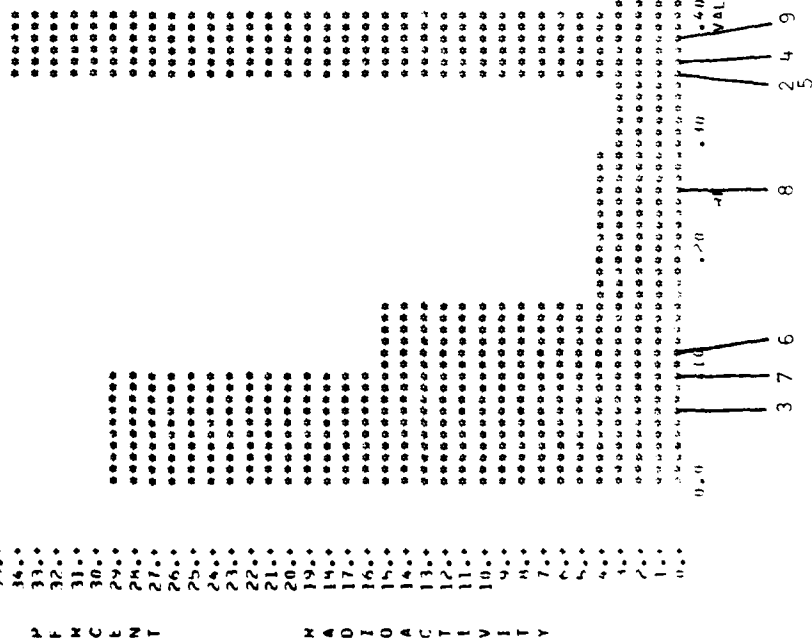
Figure 32-E4: Solvent IX

	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

2 3 4 5 6 7

RADIOACTIVITY

Figure 32-E₅: Solvent I

[illegible]Figure 32-E₅: Solvent IX

SOLVENT I Not

wt. %	wt. %	wt. %	wt. %
0.0000	154.0	0.0	0.0
0.0436	70.0	0.1	0.1
0.1563	73.4	0.3	0.3
0.2188	77.5	0.4	0.4
0.2414	80.9	0.4	0.4
0.3437	85.2	0.5	0.5
0.4063	114.4	0.6	0.6
0.4688	197.2	0.7	0.7
0.5313	220.7	0.8	0.8
0.5938	320.0	1.2	1.2
0.6563	450.6	1.6	1.6
0.7188	684.4	2.5	2.5
0.7813	14237.1	51.6	51.6
0.8438	8259.0	30.0	30.0
0.9063	1958.4	7.1	7.1
0.9688	578.0	2.1	2.1
1.0	99.2	0.0	0.0

P F R C E F N T

N A D I O A C T I V I T Y

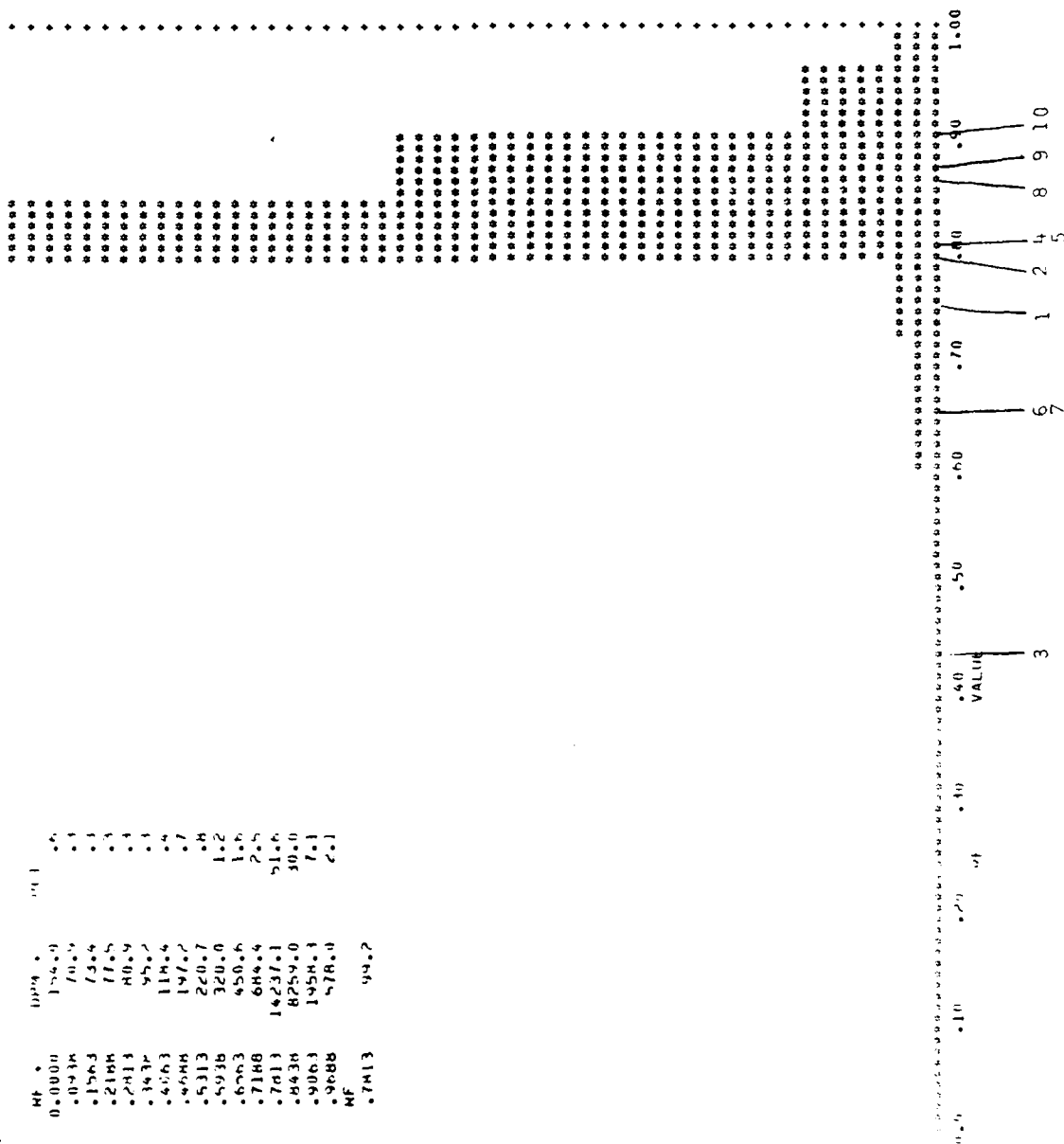


Figure 32-E₆: Solvent I

SOLVENT - Nitro

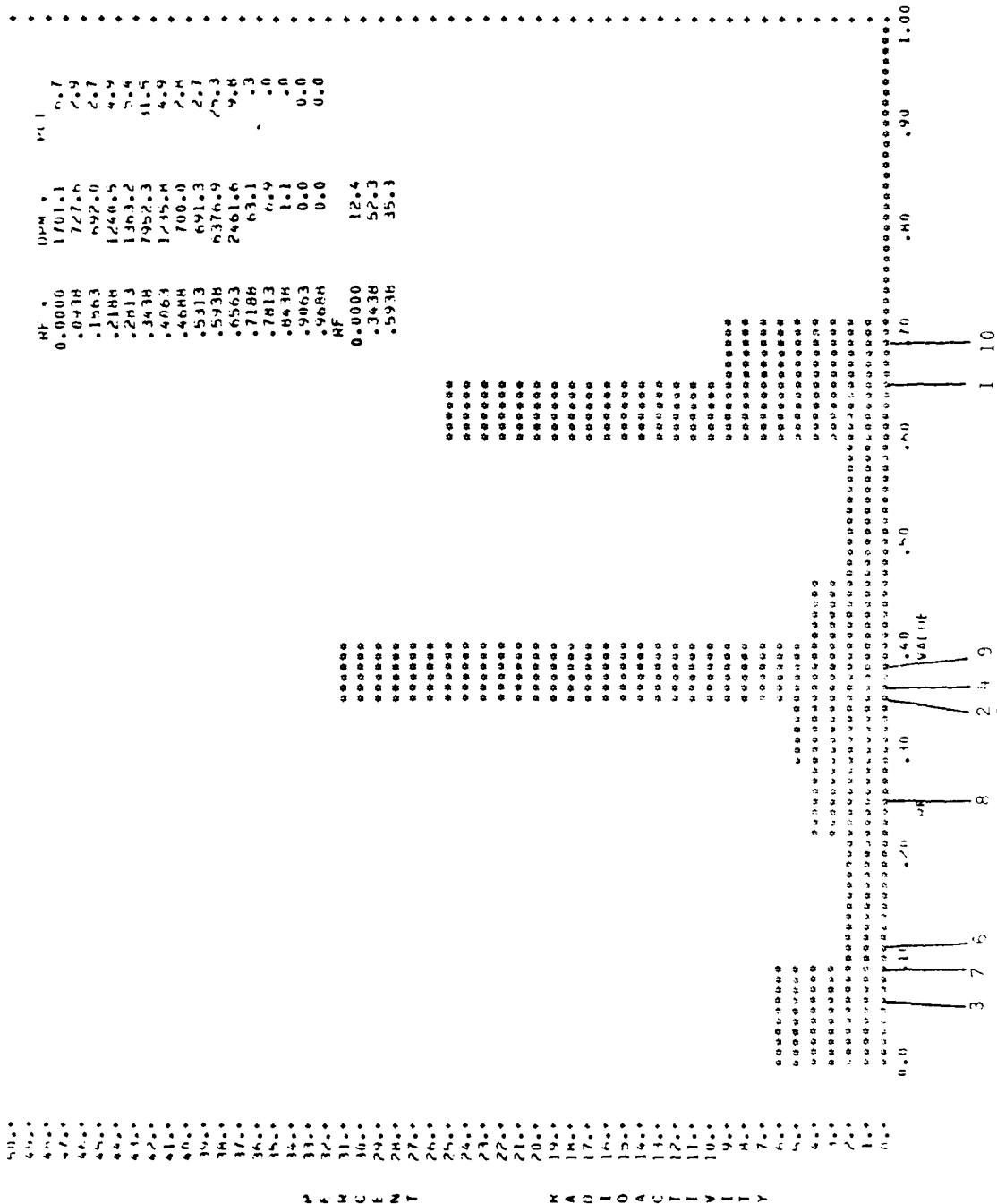


Figure 32-E₆: Solvent IX

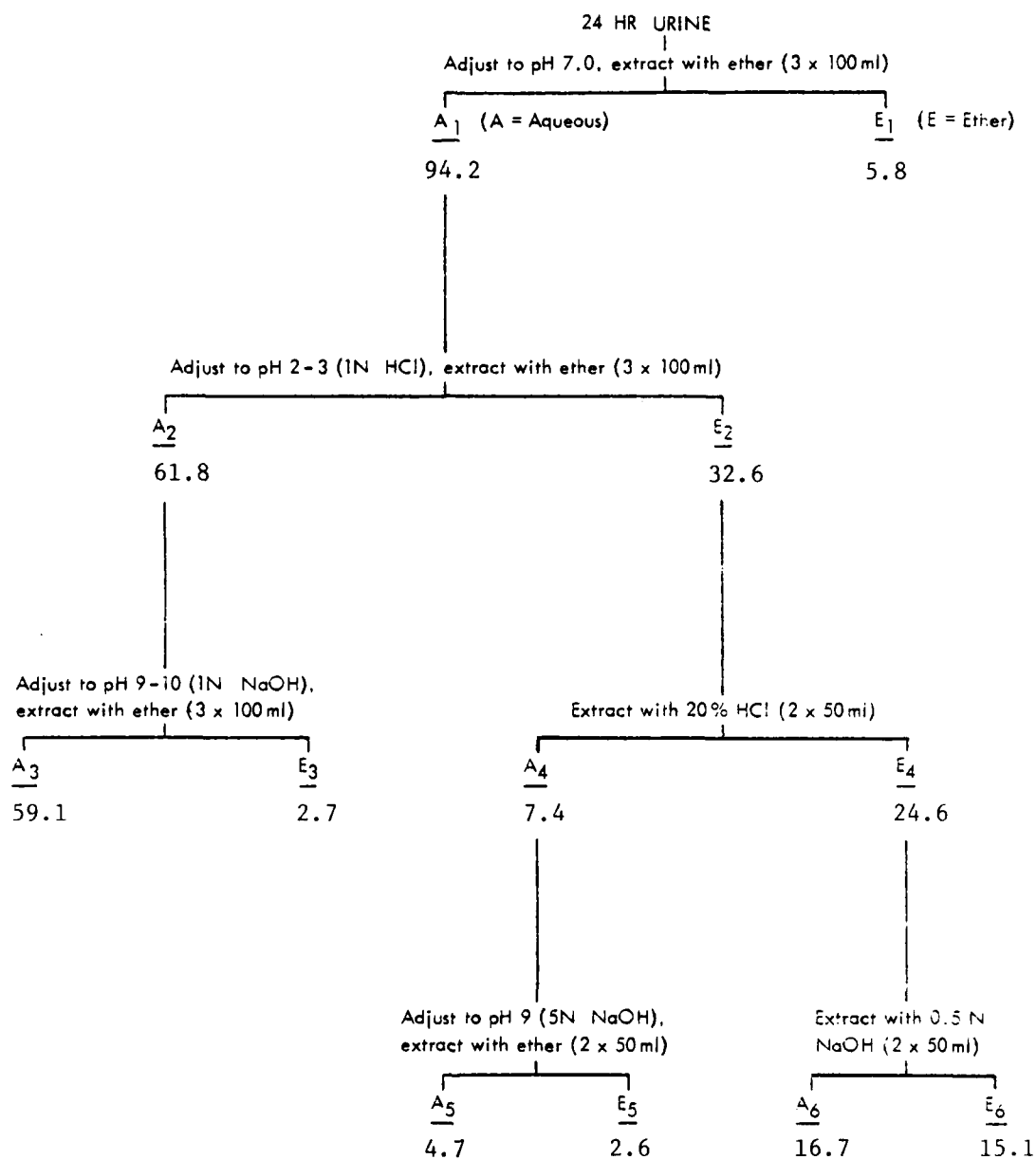


Figure 33: Fractionation of 24-Hr Urine Obtained from Rabbits Treated Orally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 34, E₁-6₆: TLC of Ether-Extractable Products Obtained from 24-Hr Urine of Rabbits Treated Orally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid: water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. (For reference metabolites (1-10) see Figure 26 or Table 19.) Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 34 follows

NO. 34-E1: Solvent I

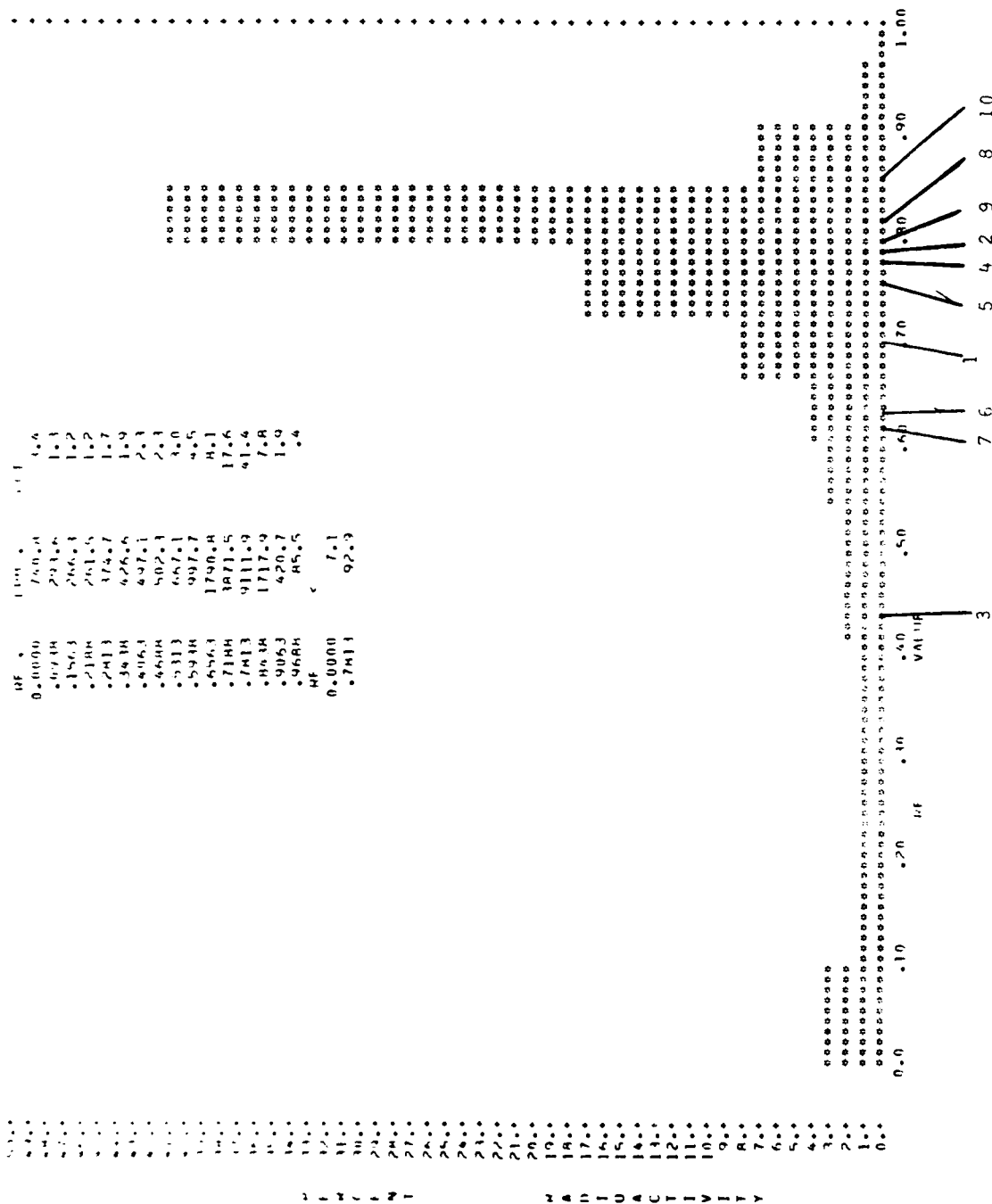


Figure 34-E1: Solvent I

SOLVENT + R₂ SAQ[1] 11

50.0	0.00000	10.434.1	0.2.9	.
49.0	0.038	27.99.1	11.2	.
48.0	0.100	15.89.5	6.6	.
47.0	0.200	9.76.9	4.0	.
46.0	0.300	12.92.1	3.3	.
45.0	0.400	20.67.9	0.6	.
44.0	0.500	46.89.3	17.0	.
43.0	0.600	26.12.3	1.1	.
42.0	0.700	15.7.1	6	.
41.0	0.800	22.5.7	0	.
40.0	0.900	05.2	0	.
39.0	1.000	12.0	0	.
38.0	1.100	1.2	0	.
37.0	0.038	0.0	0	.
36.0	0.100	0.0	0	.
35.0	0.200	1.2	0	.
34.0	0.300	0.0	0	.
33.0	0.400	0.0	0	.
32.0	0.500	0.0	0	.
31.0	0.600	0.0	0	.
30.0	0.700	0.0	0	.
29.0	0.800	0.0	0	.
28.0	0.900	0.0	0	.
27.0	1.000	0.0	0	.
26.0	0.038	0.0	0	.
25.0	0.100	0.0	0	.
24.0	0.200	0.0	0	.
23.0	0.300	0.0	0	.
22.0	0.400	0.0	0	.
21.0	0.500	0.0	0	.
20.0	0.600	0.0	0	.
19.0	0.700	0.0	0	.
18.0	0.800	0.0	0	.
17.0	0.900	0.0	0	.
16.0	1.000	0.0	0	.
15.0	0.038	0.0	0	.
14.0	0.100	0.0	0	.
13.0	0.200	0.0	0	.
12.0	0.300	0.0	0	.
11.0	0.400	0.0	0	.
10.0	0.500	0.0	0	.
9.0	0.600	0.0	0	.
8.0	0.700	0.0	0	.
7.0	0.800	0.0	0	.
6.0	0.900	0.0	0	.
5.0	1.000	0.0	0	.
4.0	0.038	0.0	0	.
3.0	0.100	0.0	0	.
2.0	0.200	0.0	0	.
1.0	0.300	0.0	0	.
0.0	0.400	0.0	0	.

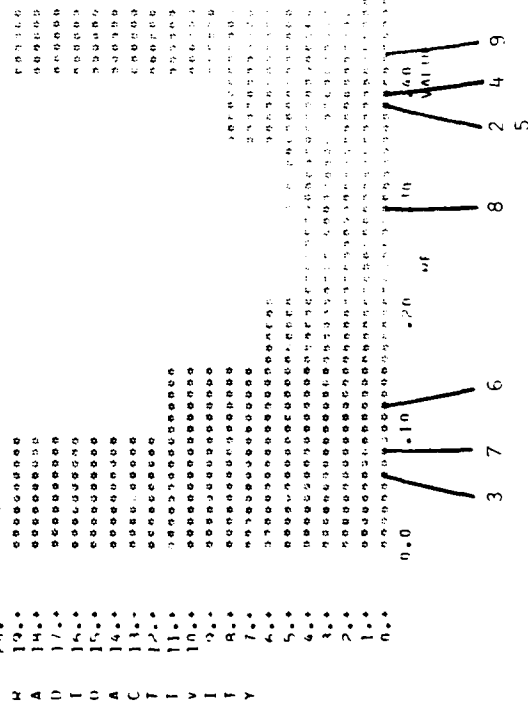
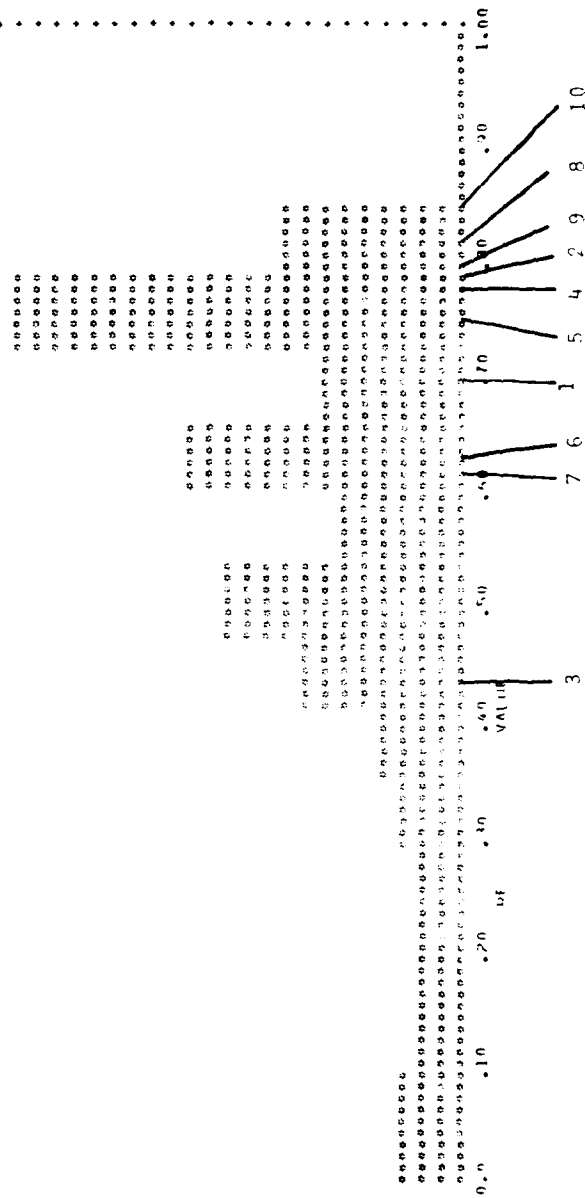


Figure 34-E1: Solvent IX

[illegible]

DE	100-24	1-1
0.0000	4600.00	46.2
0.0034	3131.00	31.3
0.0068	2456.00	24.6
0.0102	1781.00	17.8
0.0136	1106.00	11.1
0.0170	831.00	8.3
0.0204	556.00	5.6
0.0238	281.00	2.8
0.0272	106.00	1.1
0.0306	31.00	0.3
0.0340	6.00	0.6
0.0374	1.00	0.1
0.0408	0.00	0.0
0.0442	0.00	0.0
0.0476	0.00	0.0
0.0510	0.00	0.0
0.0544	0.00	0.0
0.0578	0.00	0.0
0.0612	0.00	0.0
0.0646	0.00	0.0
0.0680	0.00	0.0
0.0714	0.00	0.0
0.0748	0.00	0.0
0.0782	0.00	0.0
0.0816	0.00	0.0
0.0850	0.00	0.0
0.0884	0.00	0.0
0.0918	0.00	0.0
0.0952	0.00	0.0
0.0986	0.00	0.0
0.1020	0.00	0.0
0.1054	0.00	0.0
0.1088	0.00	0.0
0.1122	0.00	0.0
0.1156	0.00	0.0
0.1190	0.00	0.0
0.1224	0.00	0.0
0.1258	0.00	0.0
0.1292	0.00	0.0
0.1326	0.00	0.0
0.1360	0.00	0.0
0.1394	0.00	0.0
0.1428	0.00	0.0
0.1462	0.00	0.0
0.1496	0.00	0.0
0.1530	0.00	0.0
0.1564	0.00	0.0
0.1598	0.00	0.0
0.1632	0.00	0.0
0.1666	0.00	0.0
0.1700	0.00	0.0
0.1734	0.00	0.0
0.1768	0.00	0.0
0.1802	0.00	0.0
0.1836	0.00	0.0
0.1870	0.00	0.0
0.1904	0.00	0.0
0.1938	0.00	0.0
0.1972	0.00	0.0
0.2006	0.00	0.0
0.2040	0.00	0.0
0.2074	0.00	0.0
0.2108	0.00	0.0
0.2142	0.00	0.0
0.2176	0.00	0.0
0.2210	0.00	0.0
0.2244	0.00	0.0
0.2278	0.00	0.0
0.2312	0.00	0.0
0.2346	0.00	0.0
0.2380	0.00	0.0
0.2414	0.00	0.0
0.2448	0.00	0.0
0.2482	0.00	0.0
0.2516	0.00	0.0
0.2550	0.00	0.0
0.2584	0.00	0.0
0.2618	0.00	0.0
0.2652	0.00	0.0
0.2686	0.00	0.0
0.2720	0.00	0.0
0.2754	0.00	0.0
0.2788	0.00	0.0
0.2822	0.00	0.0
0.2856	0.00	0.0
0.2890	0.00	0.0
0.2924	0.00	0.0
0.2958	0.00	0.0
0.2992	0.00	0.0
0.3026	0.00	0.0
0.3060	0.00	0.0
0.3094	0.00	0.0
0.3128	0.00	0.0
0.3162	0.00	0.0
0.3196	0.00	0.0
0.3230	0.00	0.0
0.3264	0.00	0.0
0.3298	0.00	0.0
0.3332	0.00	0.0
0.3366	0.00	0.0
0.3400	0.00	0.0
0.3434	0.00	0.0
0.3468	0.00	0.0
0.3502	0.00	0.0
0.3536	0.00	0.0
0.3570	0.00	

Figure 34-E₂: Solvent I

SOLVENT 34-E2: Solvent IX

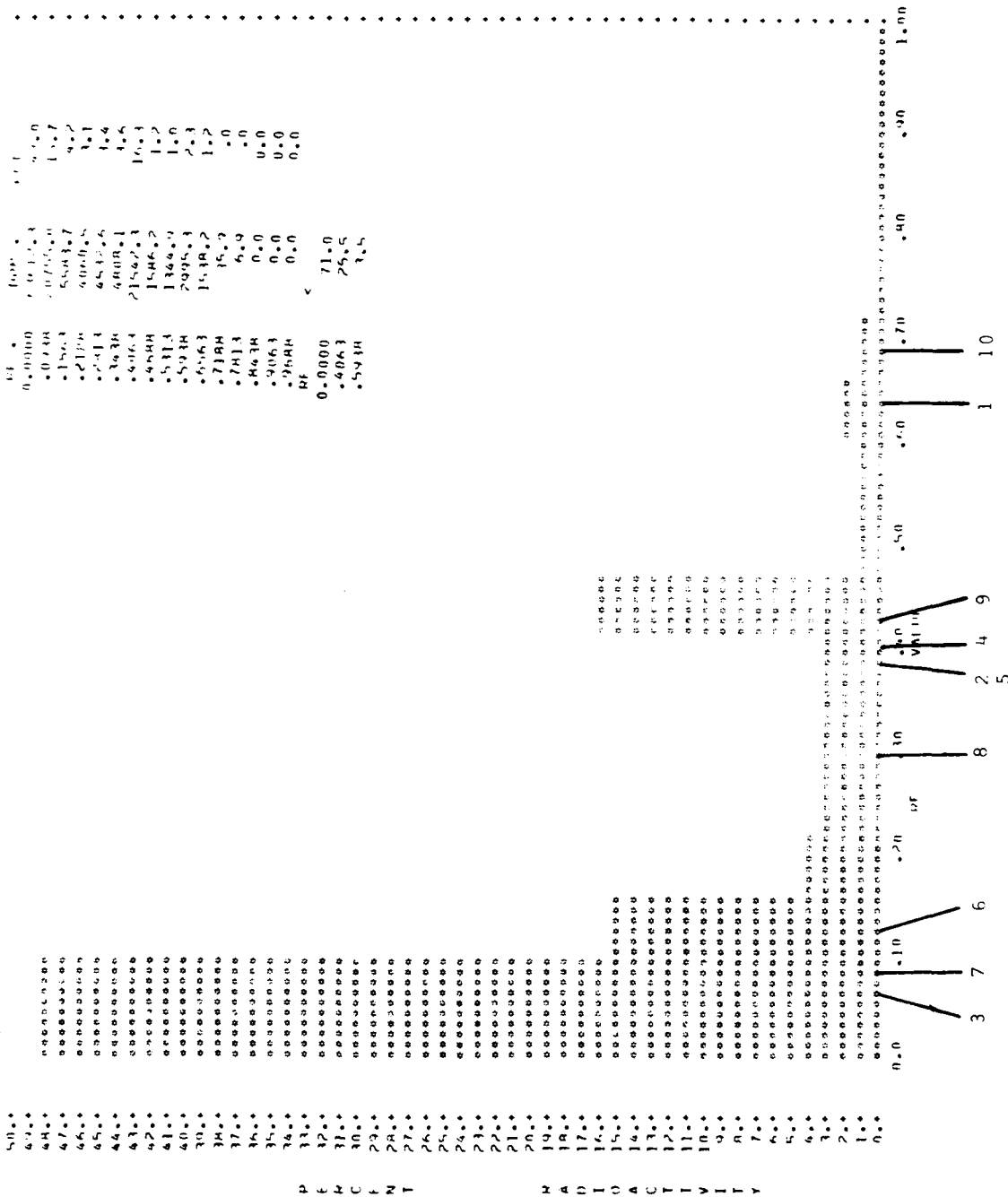


Figure 34-E2: Solvent IX

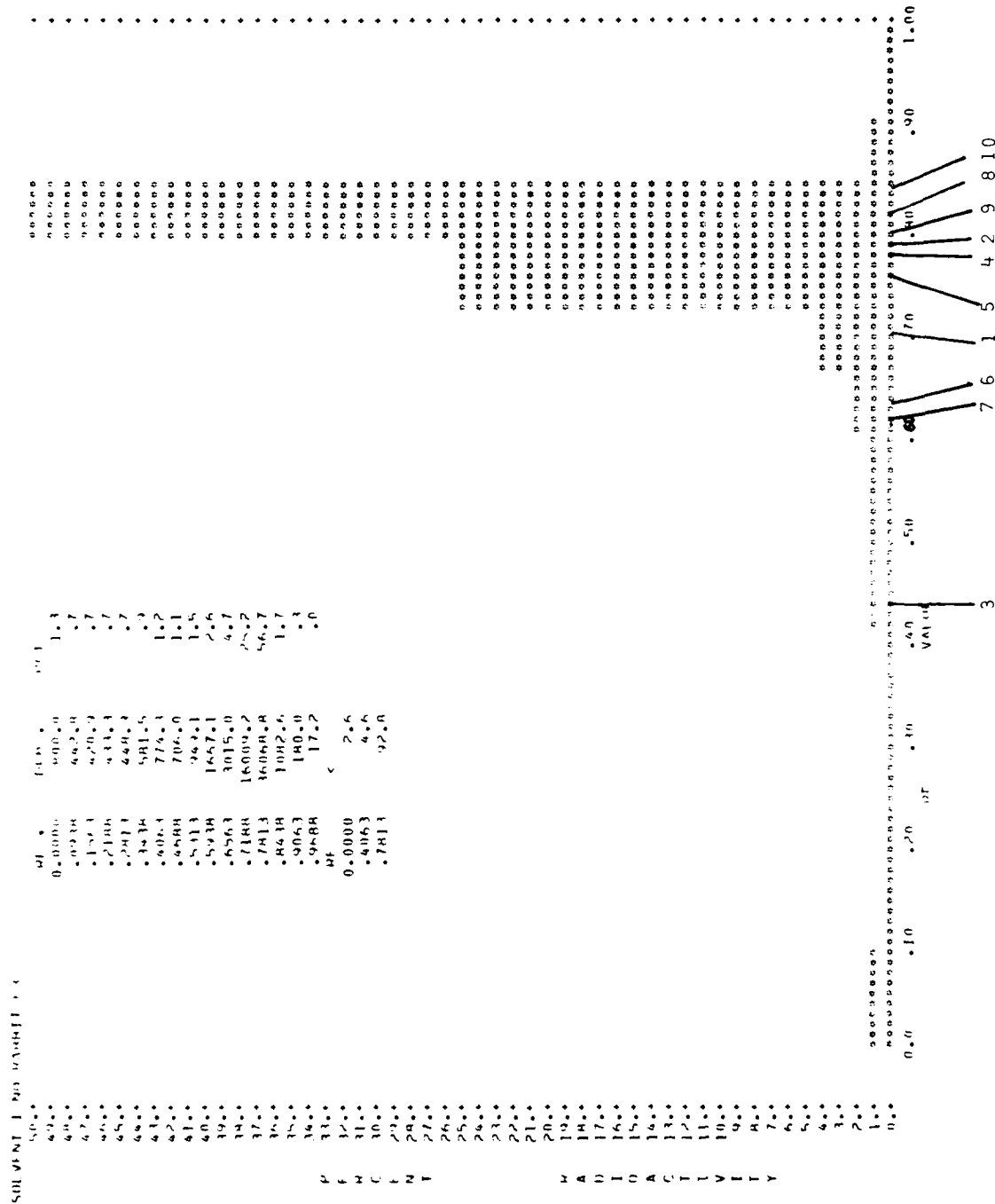


Figure 34-E3: Solvent 1

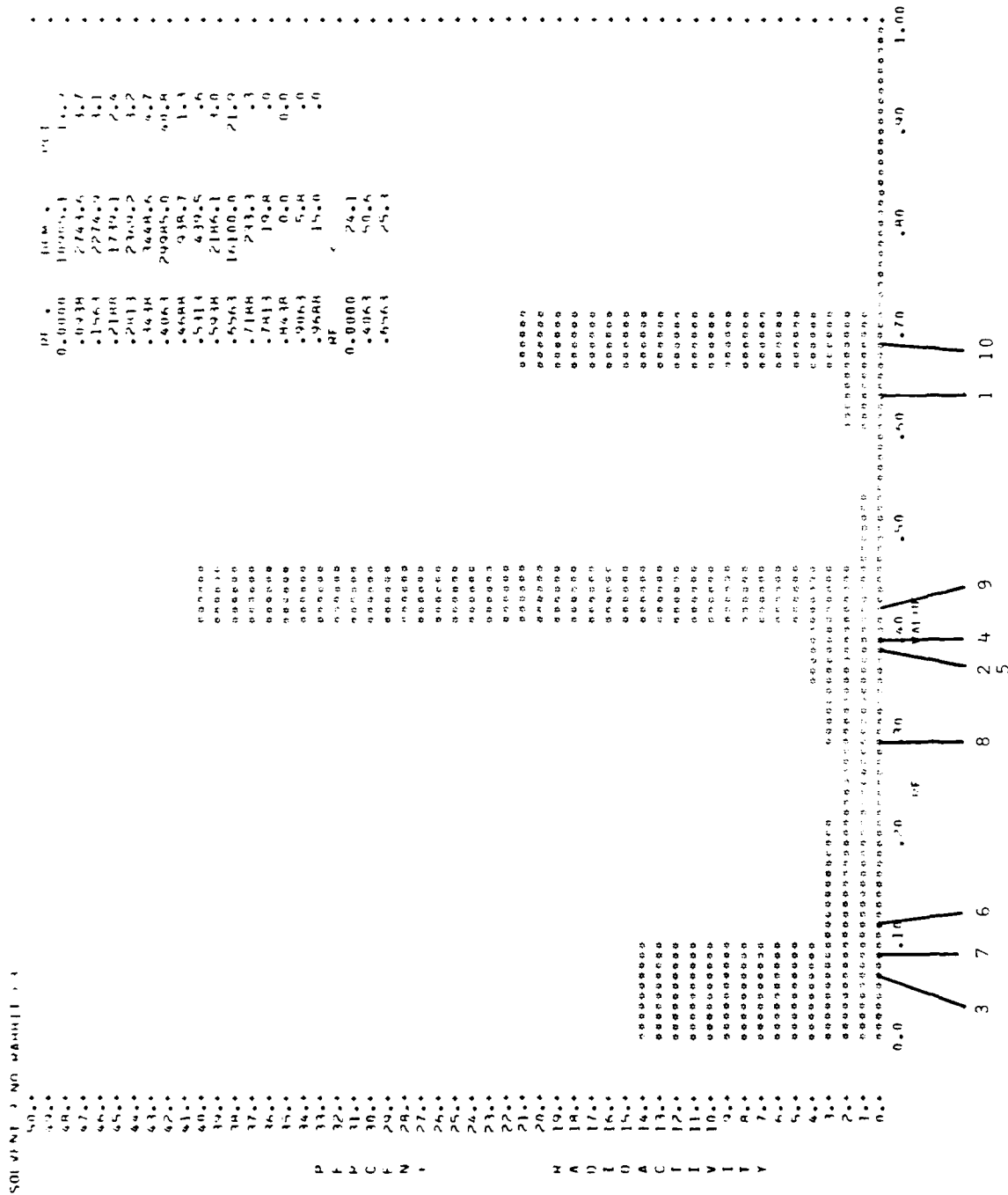


Figure 34-E3: Solvent IX

SOLVENT I NO MARK II

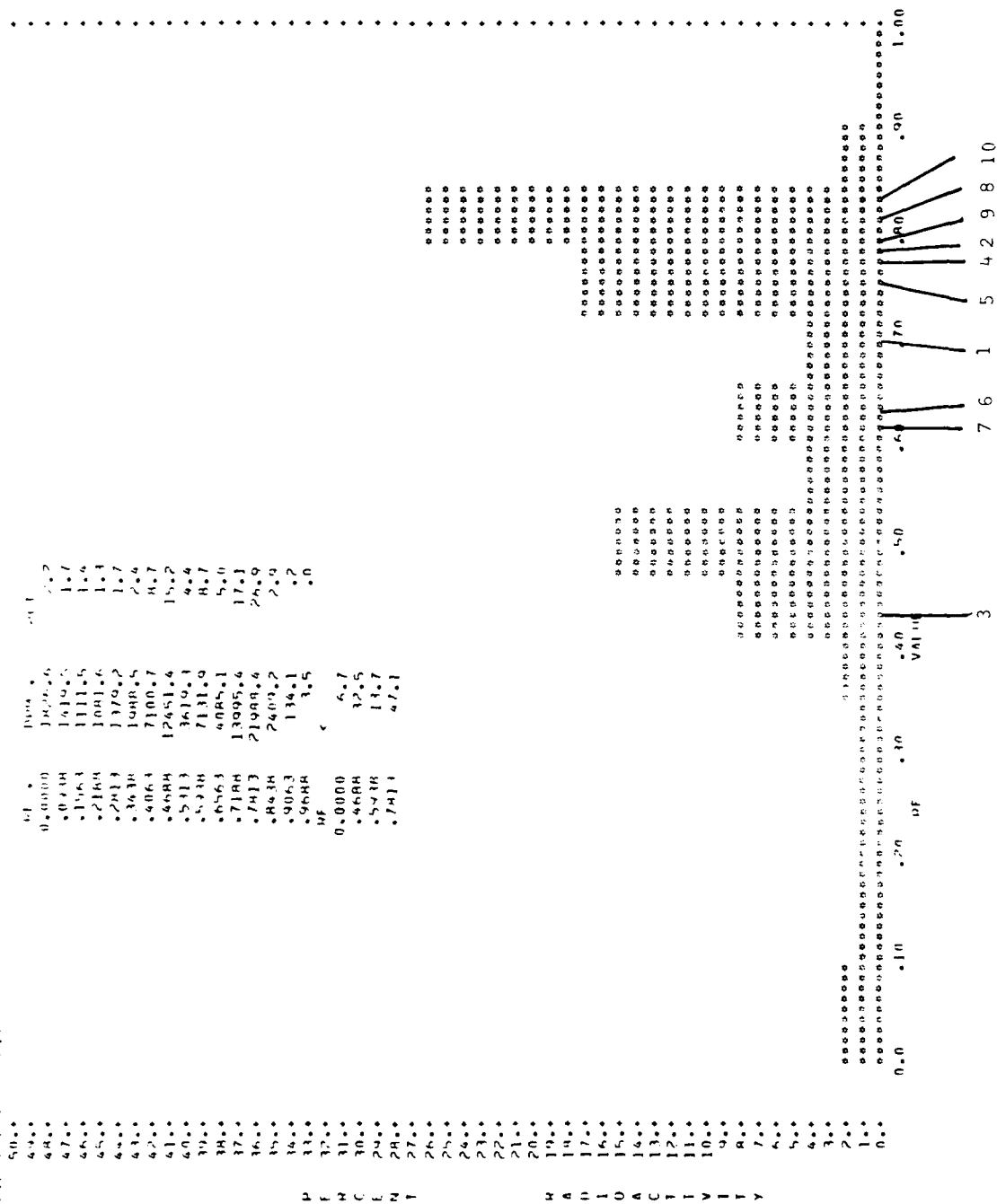
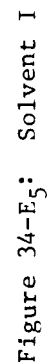


Figure 34-E₄: Solvent I





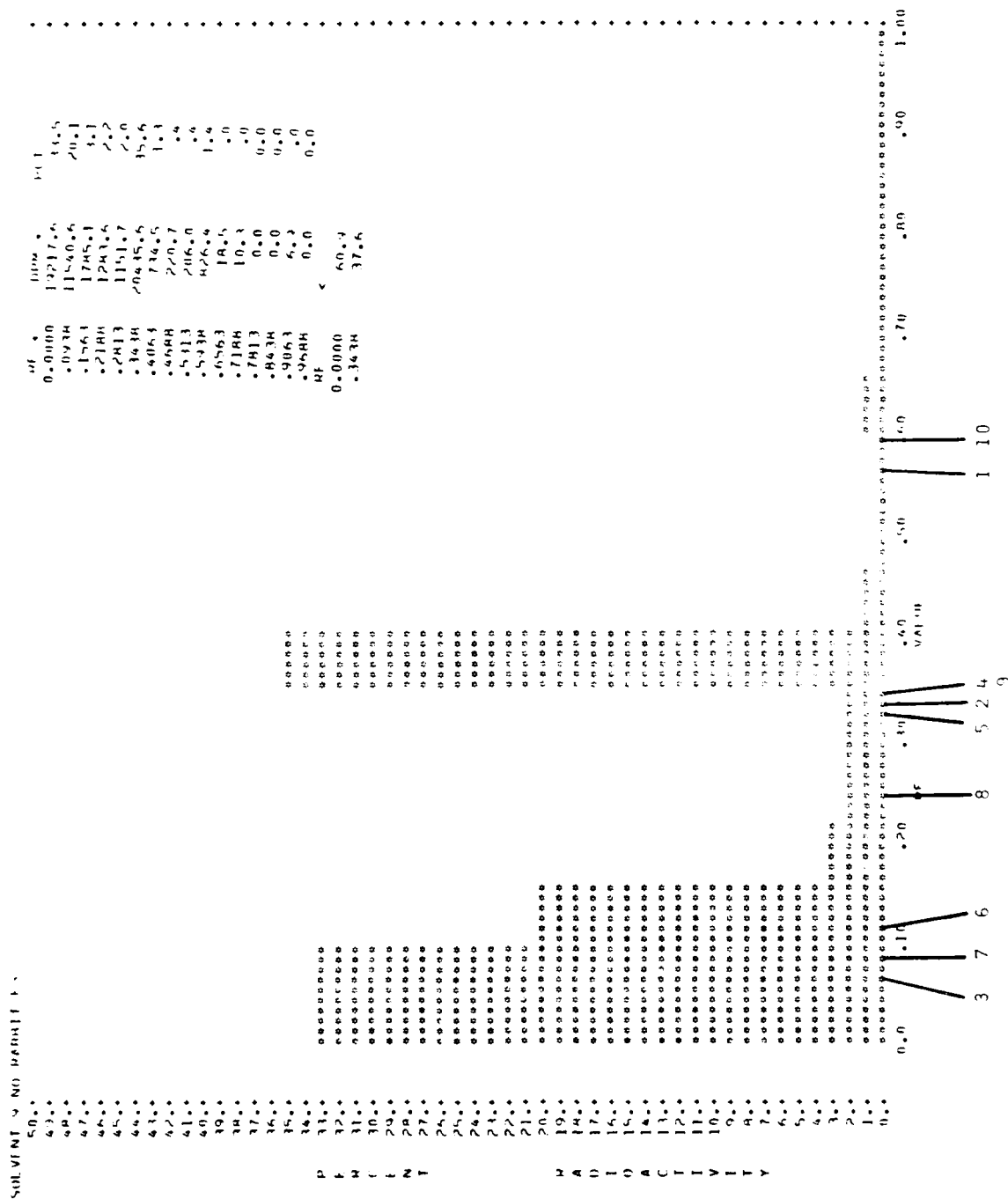


Figure 34-E₅: Solvent IX

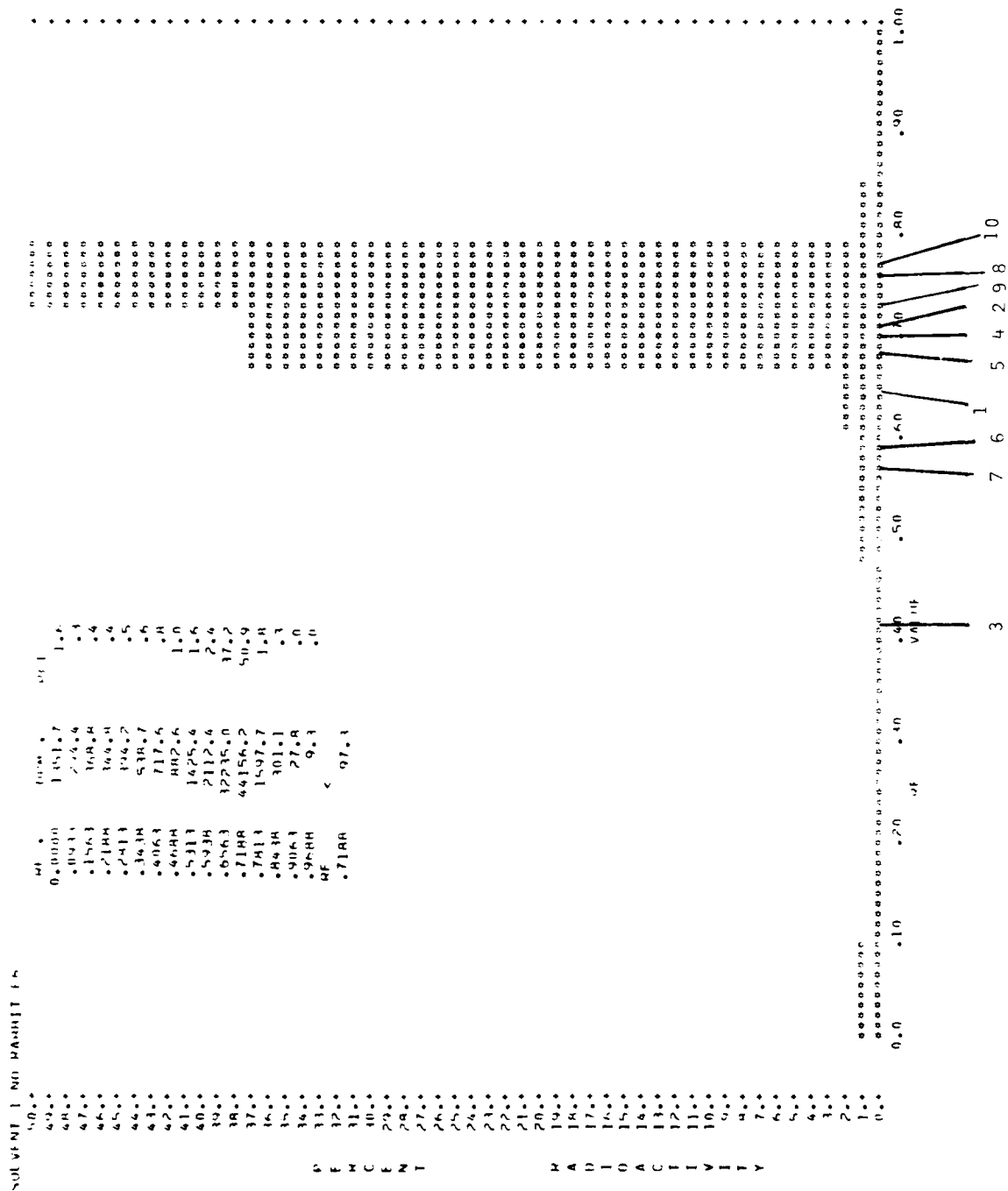
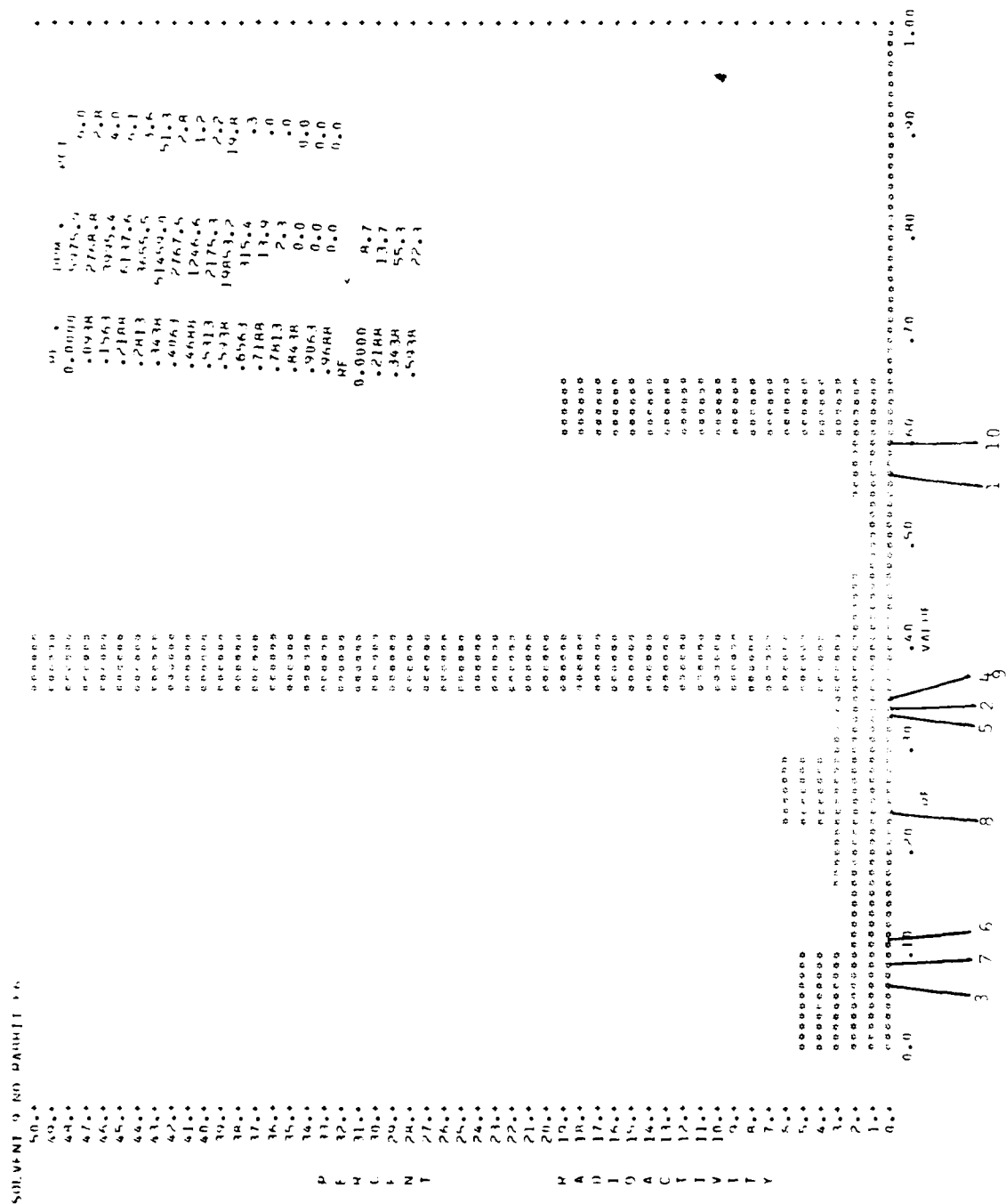


Figure 34-E₆: Solvent I



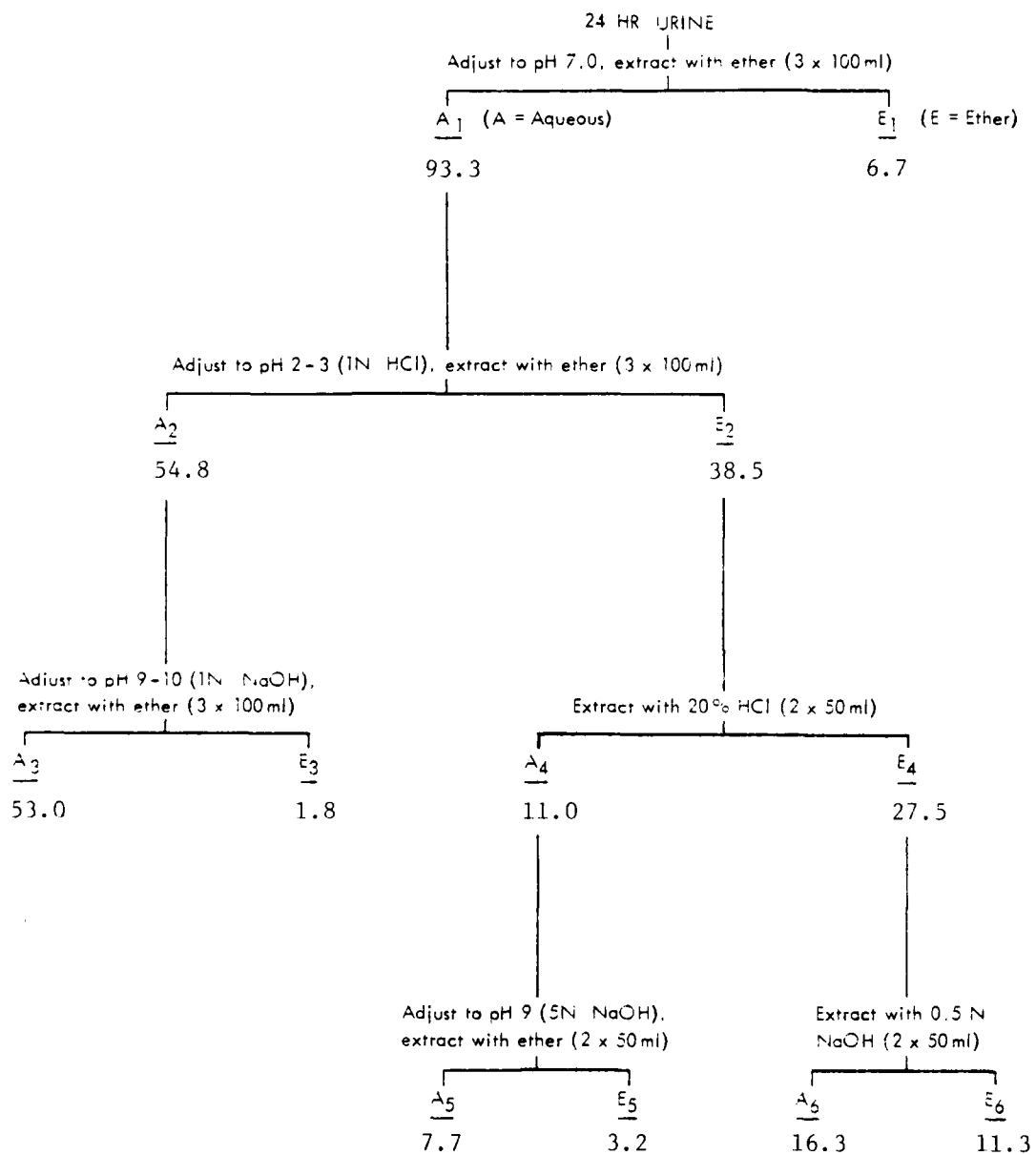


Figure 35: Fractionation of 24-Hr Urine Obtained from Rabbits Treated Dermally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 36, E₁-E₆: TLC of Ether-Extractable Products Obtained from 24-Hr Urine of Rabbits Treated Dermally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid: water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. For reference metabolites (1-10) see Figure 26 or Table 19. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 36 follows



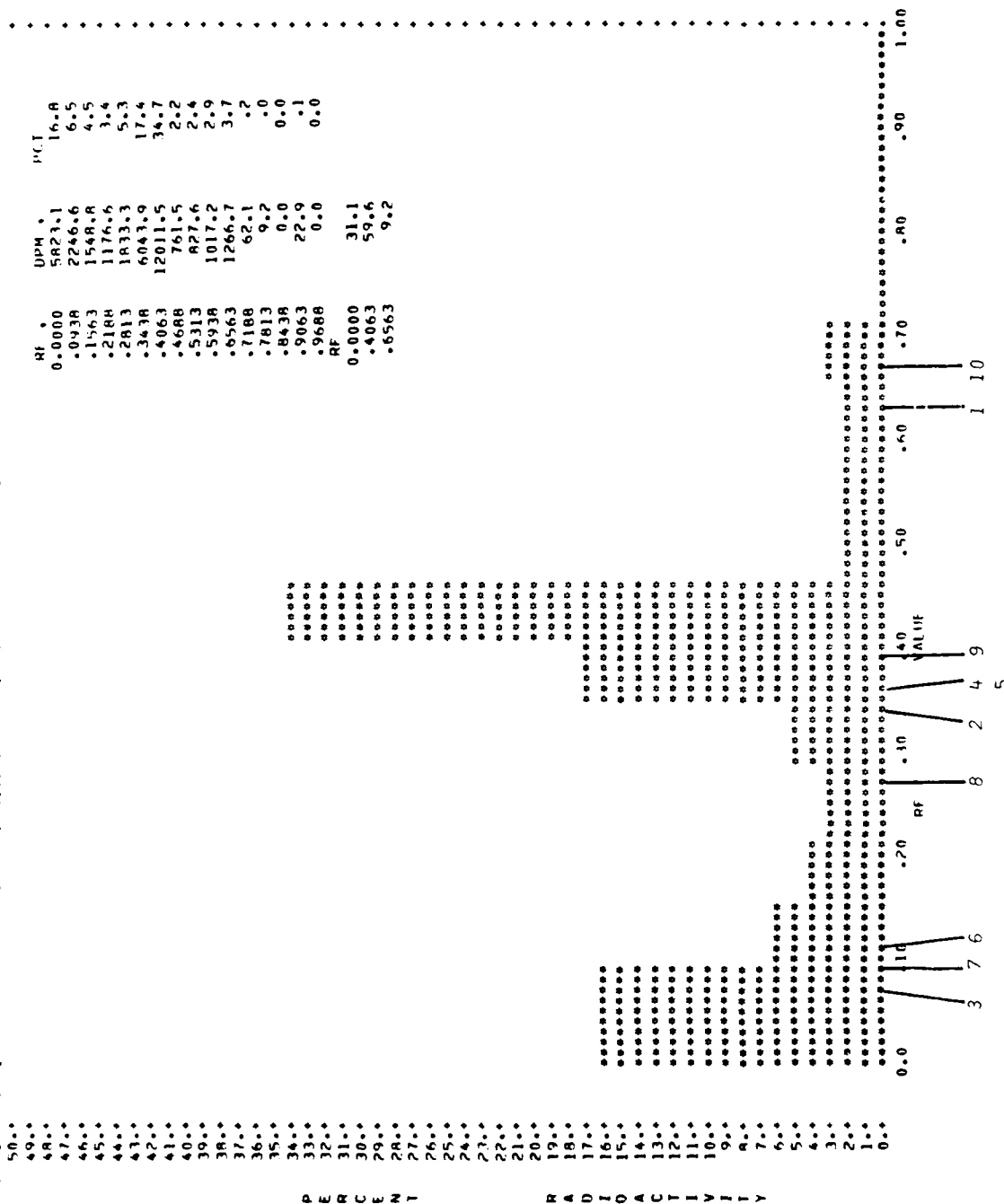
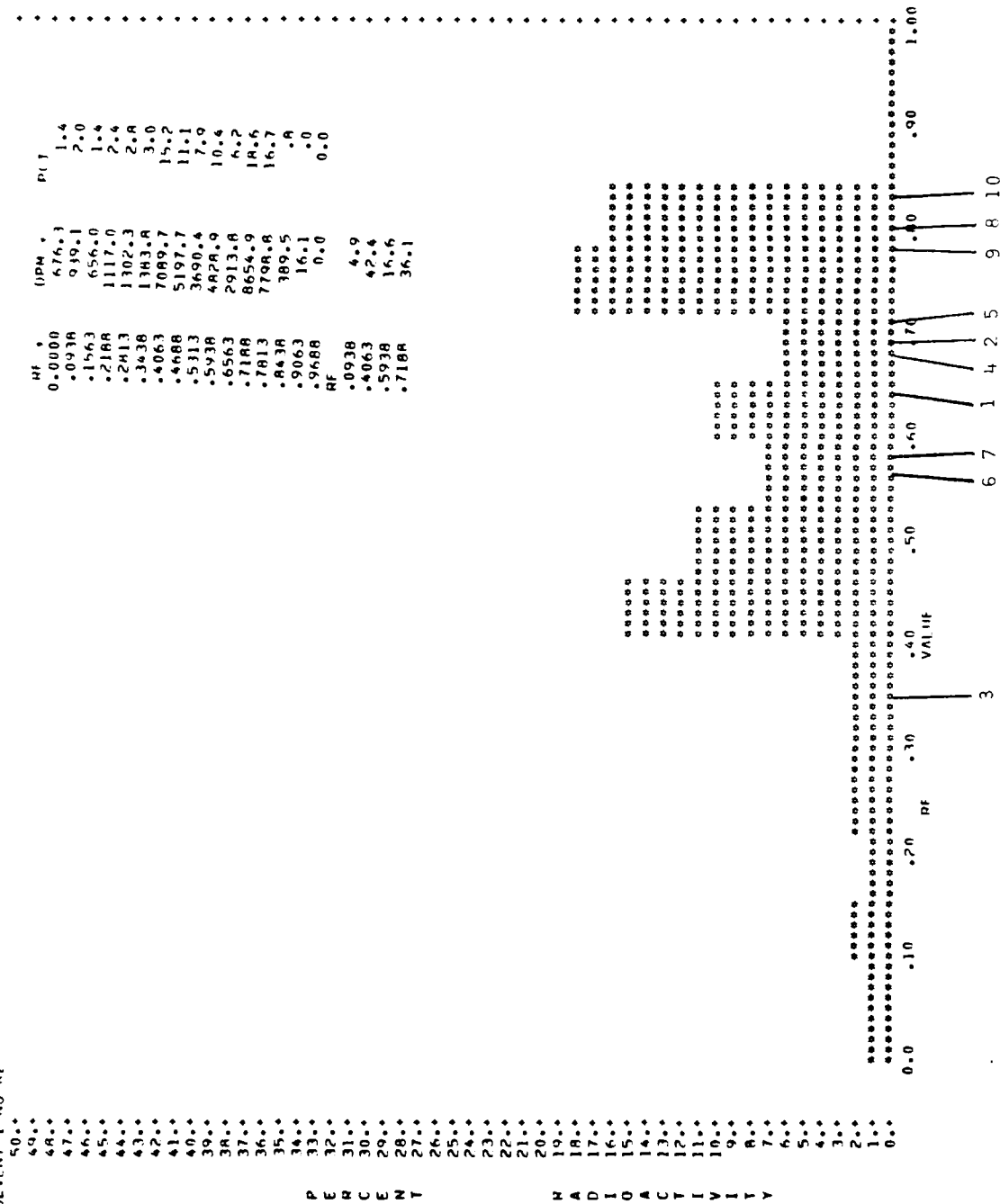


Figure 36-E₁: Solvent IX.

SOLVENT 1 NO R2



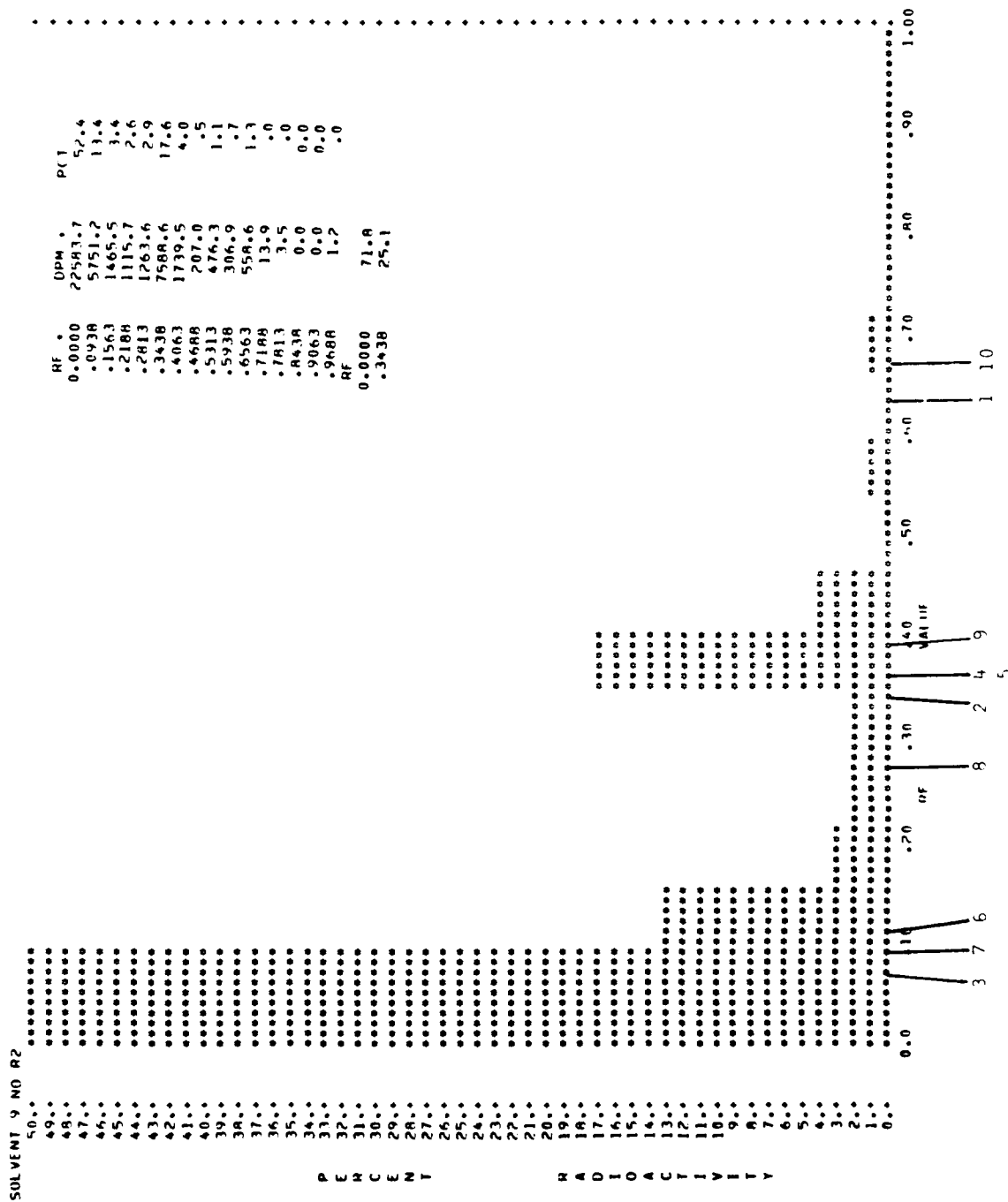


Figure 36-E₂: Solvent IX.

SOLVENT 1 NO H3

RF	DPM	PLI
50.0	257.5	2.6
49.0	125.0	1.2
48.0	87.2	.9
47.0	78.7	.8
46.0	65.1	.6
45.0	104.0	1.0
44.0	116.8	1.2
43.0	122.7	1.4
42.0	140.2	2.9
41.0	288.9	4.4
40.0	444.2	28.1
39.0	2825.9	49.6
38.0	4997.7	3.7
37.0	7813	.4
36.0	8438	0.0
35.0	9063	6.1
34.0	43.9	93.8
33.0	0.0	
32.0	RF	
31.0	0.0000	
30.0	.7813	
29.0		
28.0		
27.0		
26.0		
25.0		
24.0		
23.0		
22.0		
21.0		
20.0		
19.0		
18.0		
17.0		
16.0		
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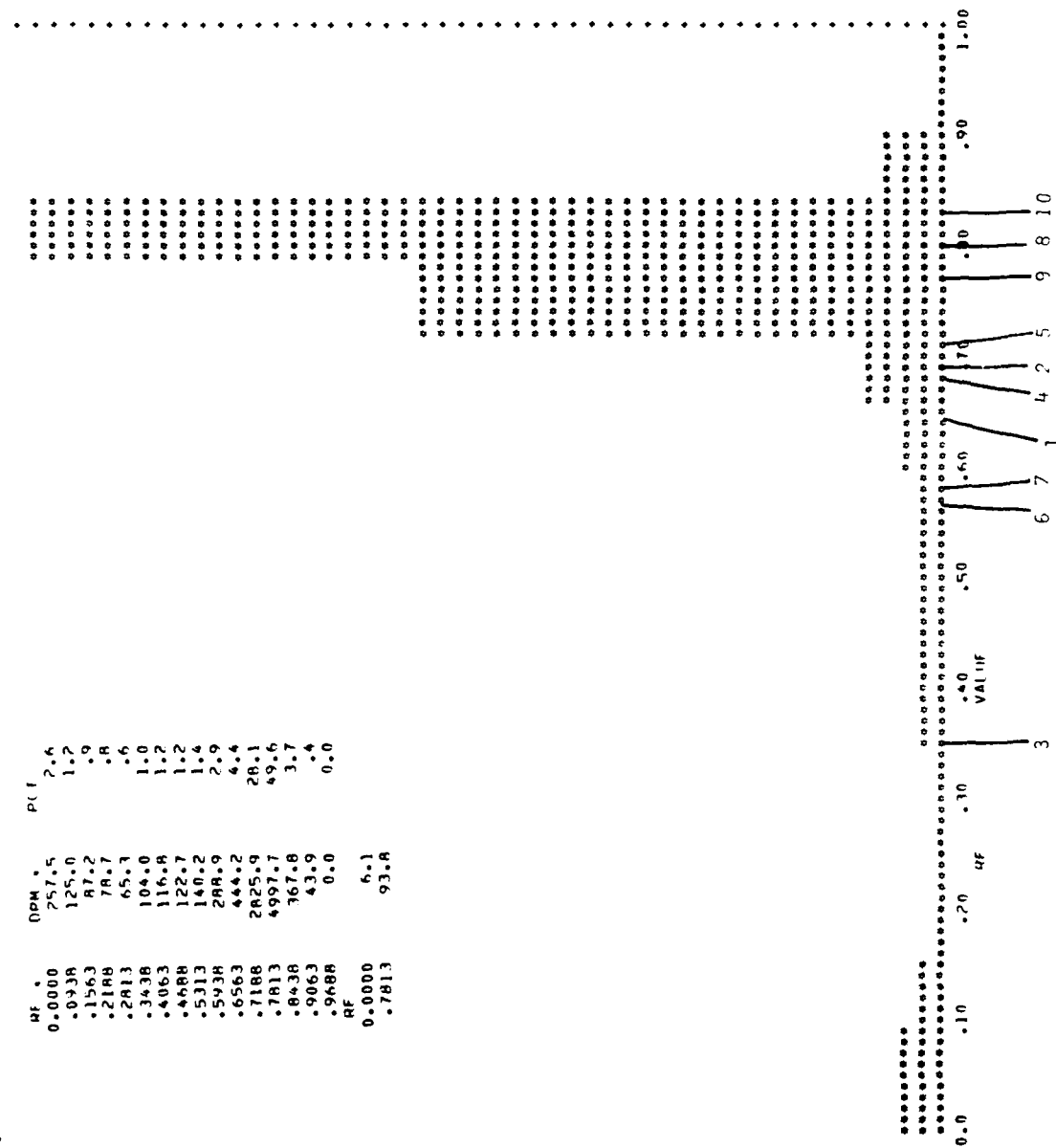


Figure 36-E₃: Solvent I.

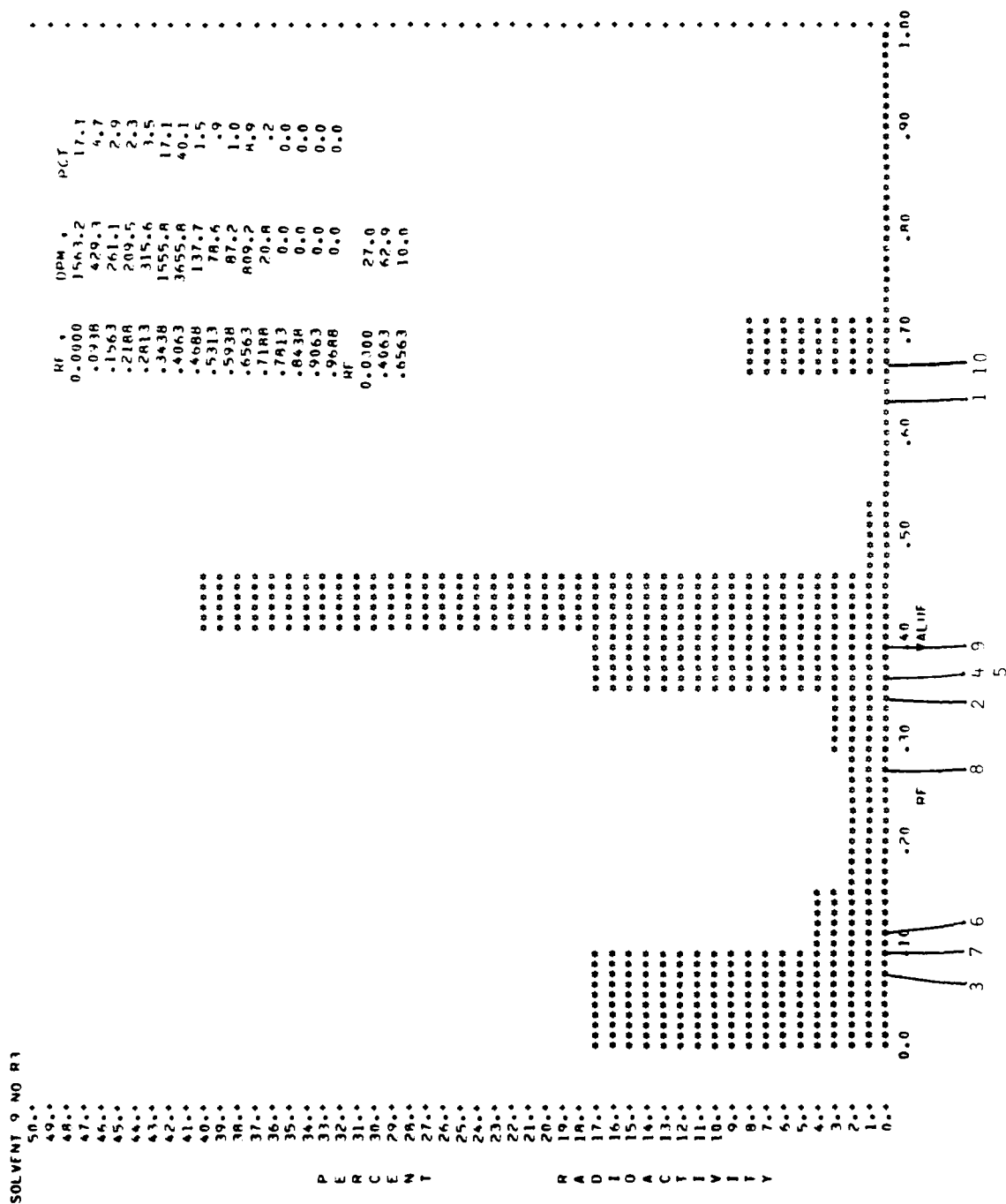


Figure 36-E₃: Solvent IX.

SOLVENT 1 NO R4

50..	0.0000	171.4	0.6
49..	.0434	262.1	1.0
48..	.1563	201.2	.8
47..	.2184	204.6	.8
46..	.2413	382.7	1.4
45..	.3434	661.6	2.5
44..	.4063	3365.8	12.6
43..	.4684	3511.5	13.1
42..	.5313	1574.1	5.9
41..	.5938	2009.2	7.5
40..	.6563	1111.0	4.2
39..	.7188	4166.7	15.6
38..	.7813	7706.9	28.8
37..	.8438	1337.6	5.0
36..	.9063	79.8	.3
35..	.9684	3.4	.0
34..	HF		
33..	.0934	2.4	
32..	.4688	36.2	
31..	.5936	11.7	
30..	.7813	49.6	

P E R C E N T

R A D I A N T

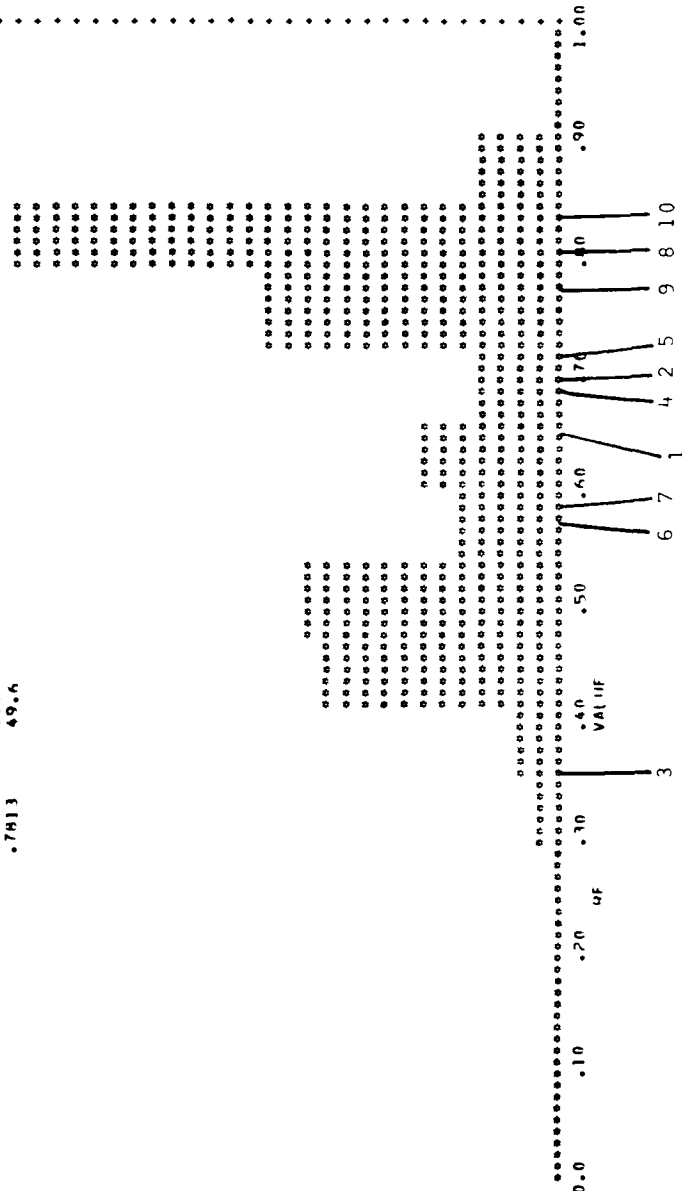


Figure 36-E₄: Solvent I.

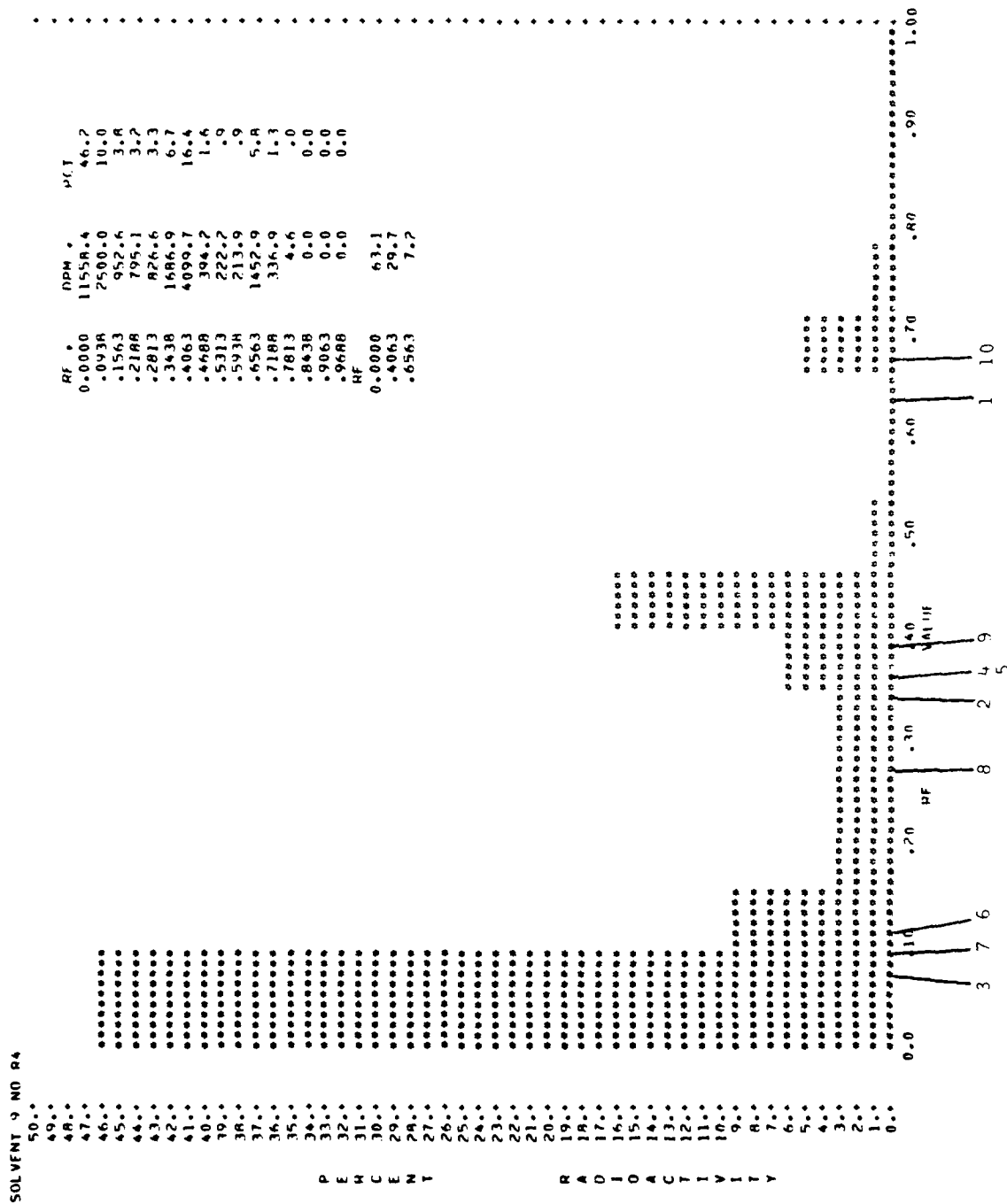


Figure 36-E₄: Solvent IX.

SOLVENT 1 NO R5

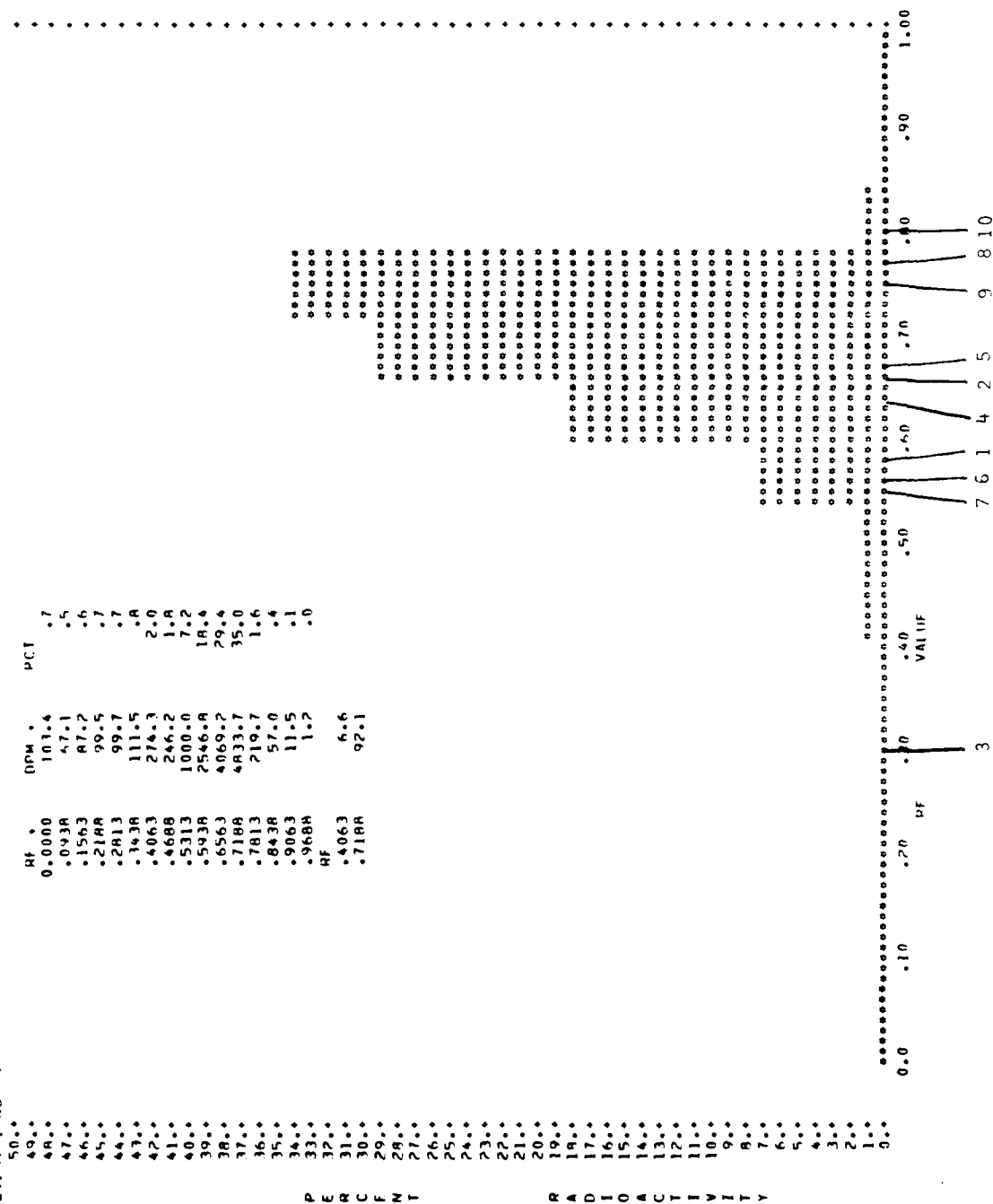


Figure 36-E₅: Solvent I.

SOLVENT 9 NO 95

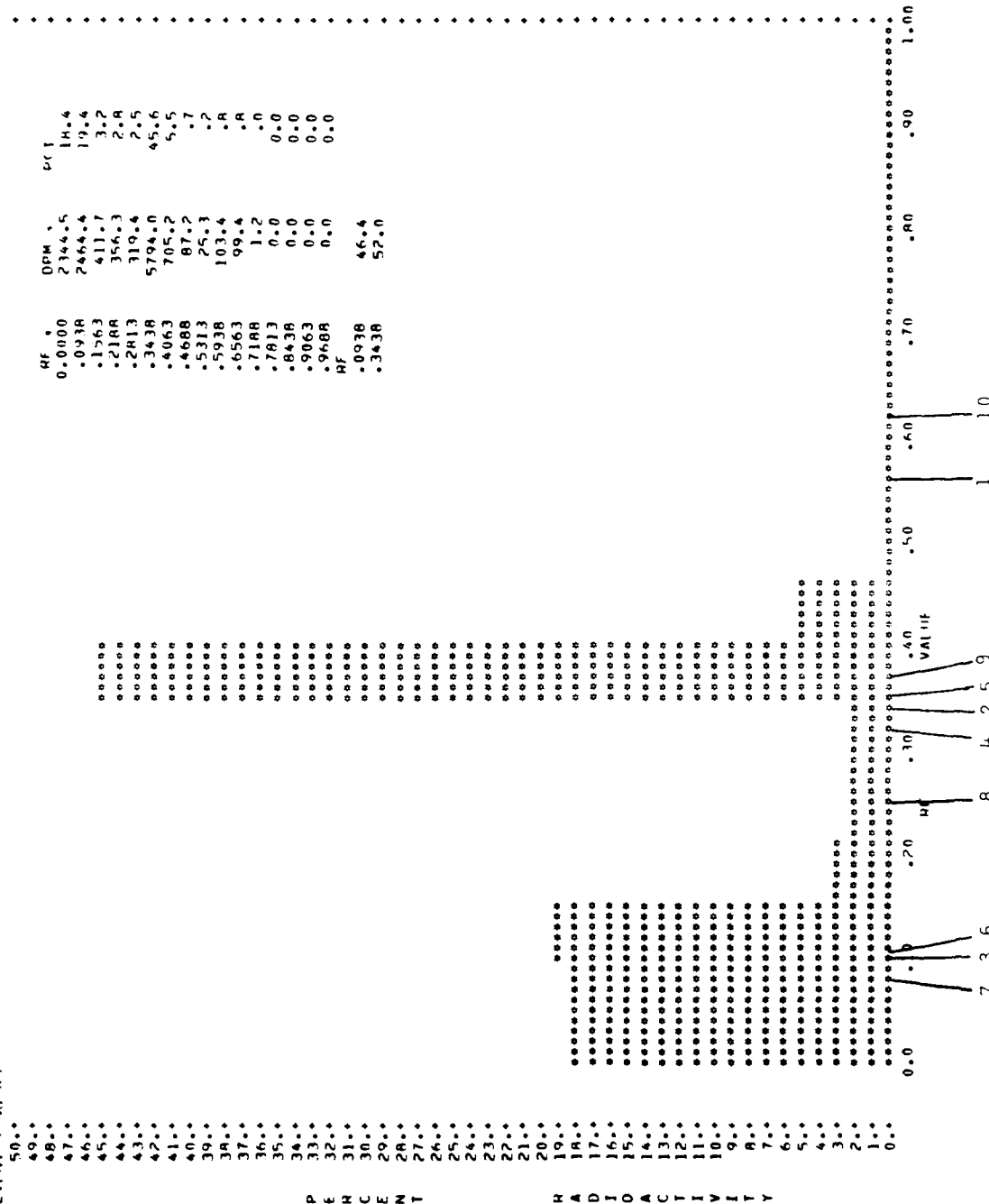


Figure 36-E₅: Solvent IX.

SOLVENT I NO R6

50..	RF	DPM	PCT
49..	0.0000	140.6	.9
48..	.0938	83.0	.2
47..	.1563	102.4	.3
46..	.2188	133.1	.4
45..	.2813	158.4	.4
44..	.3438	176.9	.9
43..	.4063	341.4	1.0
42..	.4688	396.8	1.1
41..	.5313	443.7	3.0
40..	.5938	1196.5	39.8
39..	.6563	3583.1	44.3
38..	.7188	19669.0	2.2
37..	.7813	869.0	.3
36..	.8438	120.3	.0
35..	.9063	3.4	0.0
34..	.9688	0.0	
33..	RF		
32..	.7188	98.9	
31..			
30..			
29..			
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0..			

P E H C E N T

R A D I O C I T Y

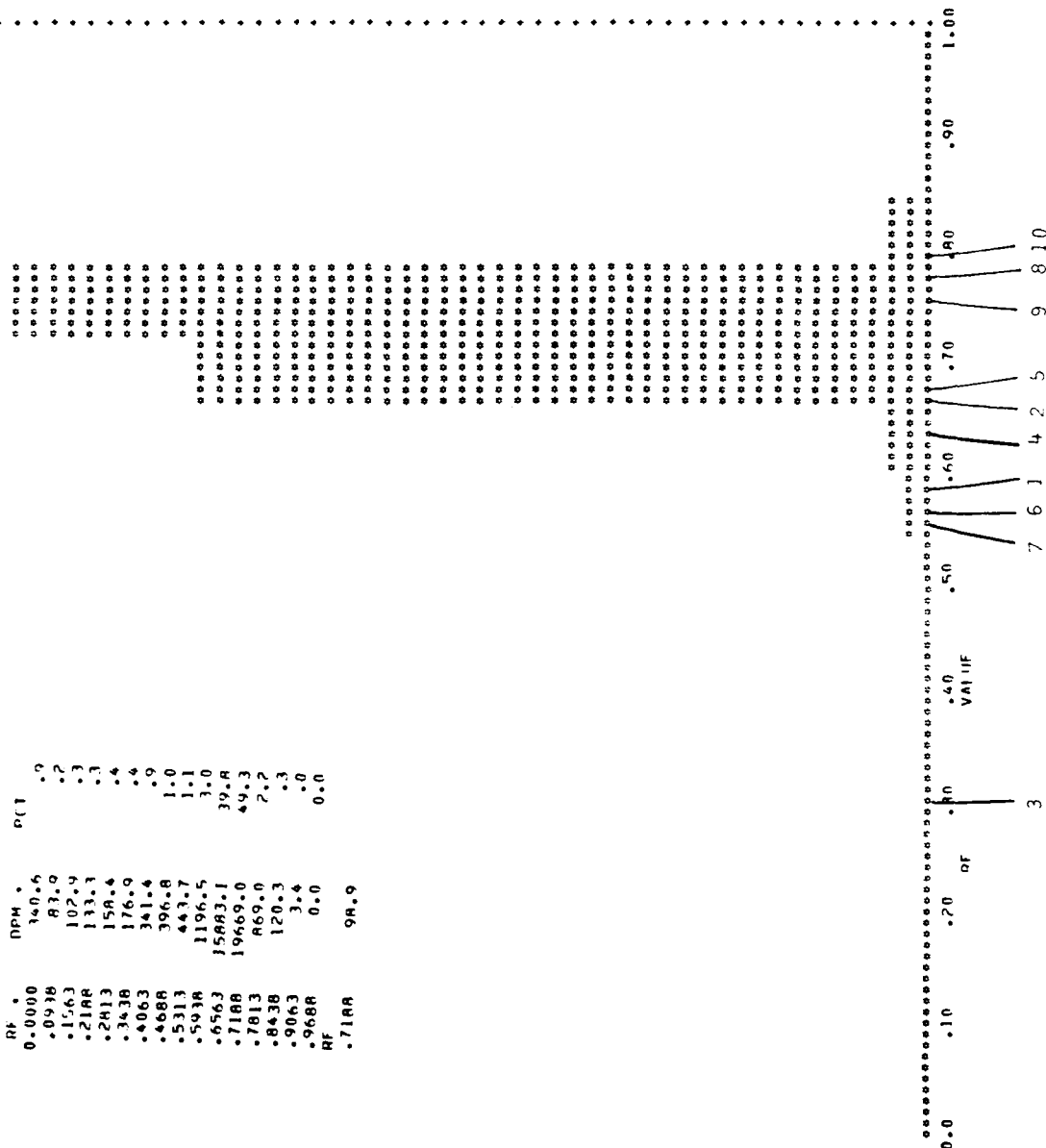


Figure 36-E₆: Solvent I.

SOLVENT 9 NO RA

50.0	0.0000	1526.6	4.1
49.0	0.0000	868.6	2.4
48.0	0.0000	1041.7	2.9
47.0	0.0000	1407.4	4.0
46.0	0.0000	1149.3	3.2
45.0	0.0000	19081.3	53.7
44.0	0.0000	1209.5	3.4
43.0	0.0000	505.8	1.4
42.0	0.0000	408.1	1.1
41.0	0.0000	4256.6	12.0
40.0	0.0000	4049.8	11.4
39.0	0.0000	23.0	1.1
38.0	0.0000	6.9	0.0
37.0	0.0000	8.38	0.0
36.0	0.0000	9063	2.3
35.0	0.0000	9688	0.0
34.0	0.0000	RF	0.0
33.0	0.0000	0.0000	6.7
32.0	0.0000	2188	10.1
31.0	0.0000	3438	59.7
30.0	0.0000	938	23.4
29.0	0.0000		
28.0	0.0000		
27.0	0.0000		
26.0	0.0000		
25.0	0.0000		
24.0	0.0000		
23.0	0.0000		
22.0	0.0000		
21.0	0.0000		
20.0	0.0000		
19.0	0.0000		
18.0	0.0000		
17.0	0.0000		
16.0	0.0000		
15.0	0.0000		
14.0	0.0000		
13.0	0.0000		
12.0	0.0000		
11.0	0.0000		
10.0	0.0000		
9.0	0.0000		
8.0	0.0000		
7.0	0.0000		
6.0	0.0000		
5.0	0.0000		
4.0	0.0000		
3.0	0.0000		
2.0	0.0000		
1.0	0.0000		

P E R C E N T

R A D I O F R E Q U E N C Y

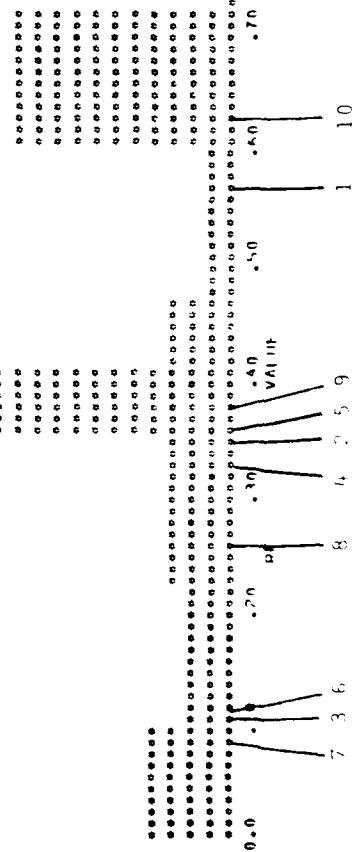


Figure 36-E₆: Solvent IX.

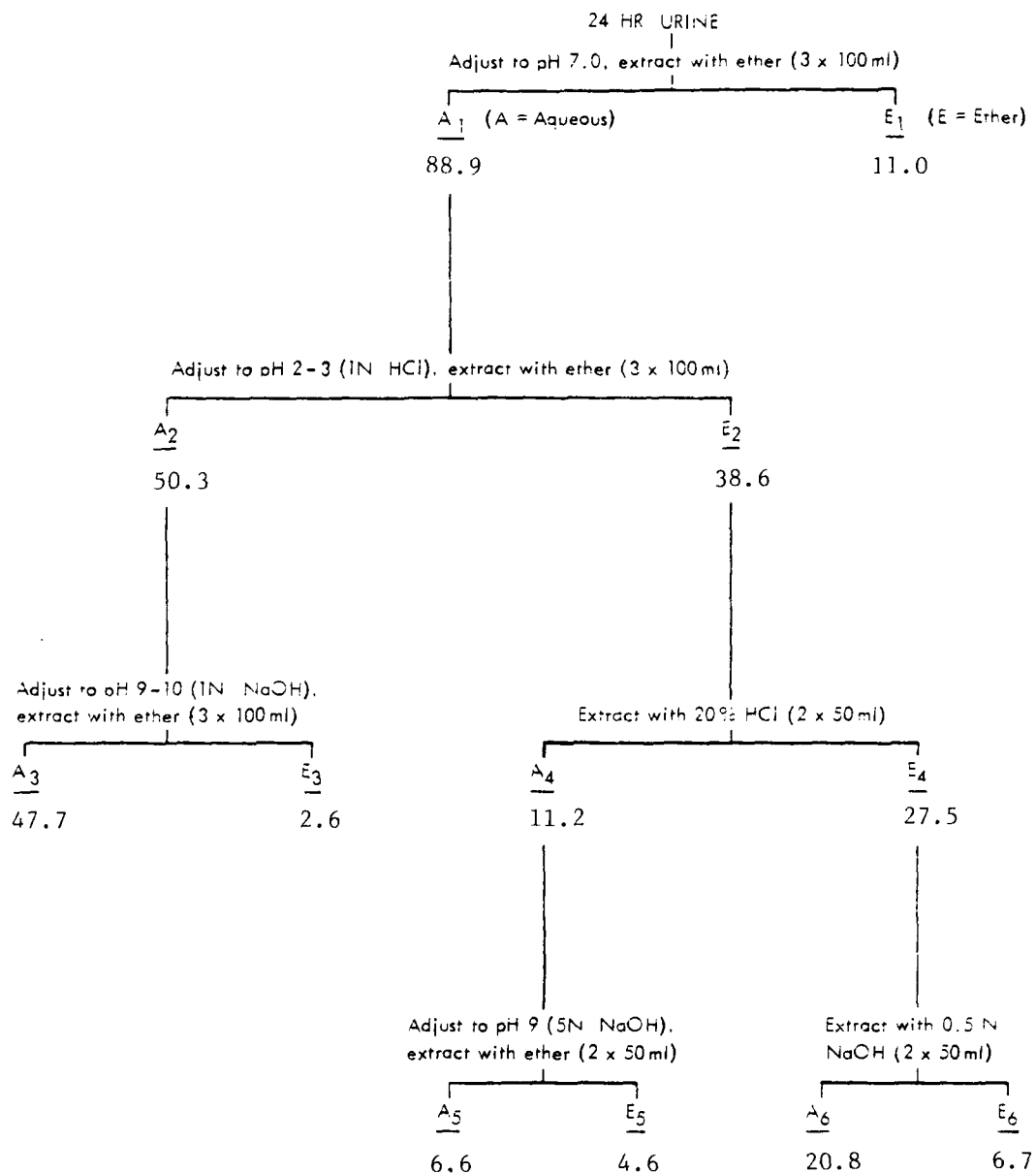


Figure 37: Fractionation of 24-Hr Urine Obtained from Dogs Treated Orally with ^{14}C -TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 38, E₁-E₆: TLC of Ether-Extractable Products Obtained from 24-Hr Urine of Dogs Treated Orally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. For reference metabolites (1-10) see Figure 26 or Table 19. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 38 follows

SOLVENT 1 NO DI

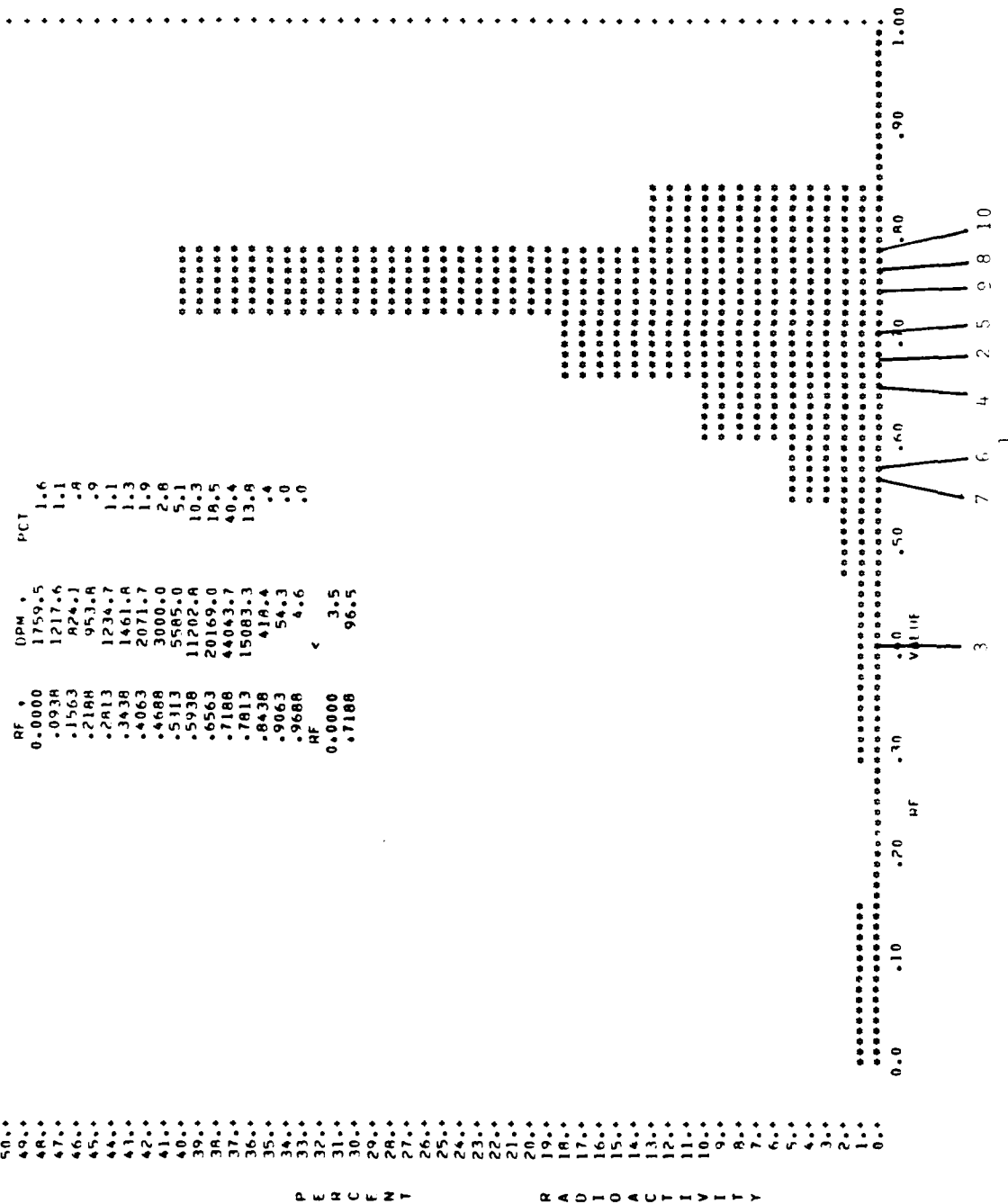


Figure 38-E1: Solvent I

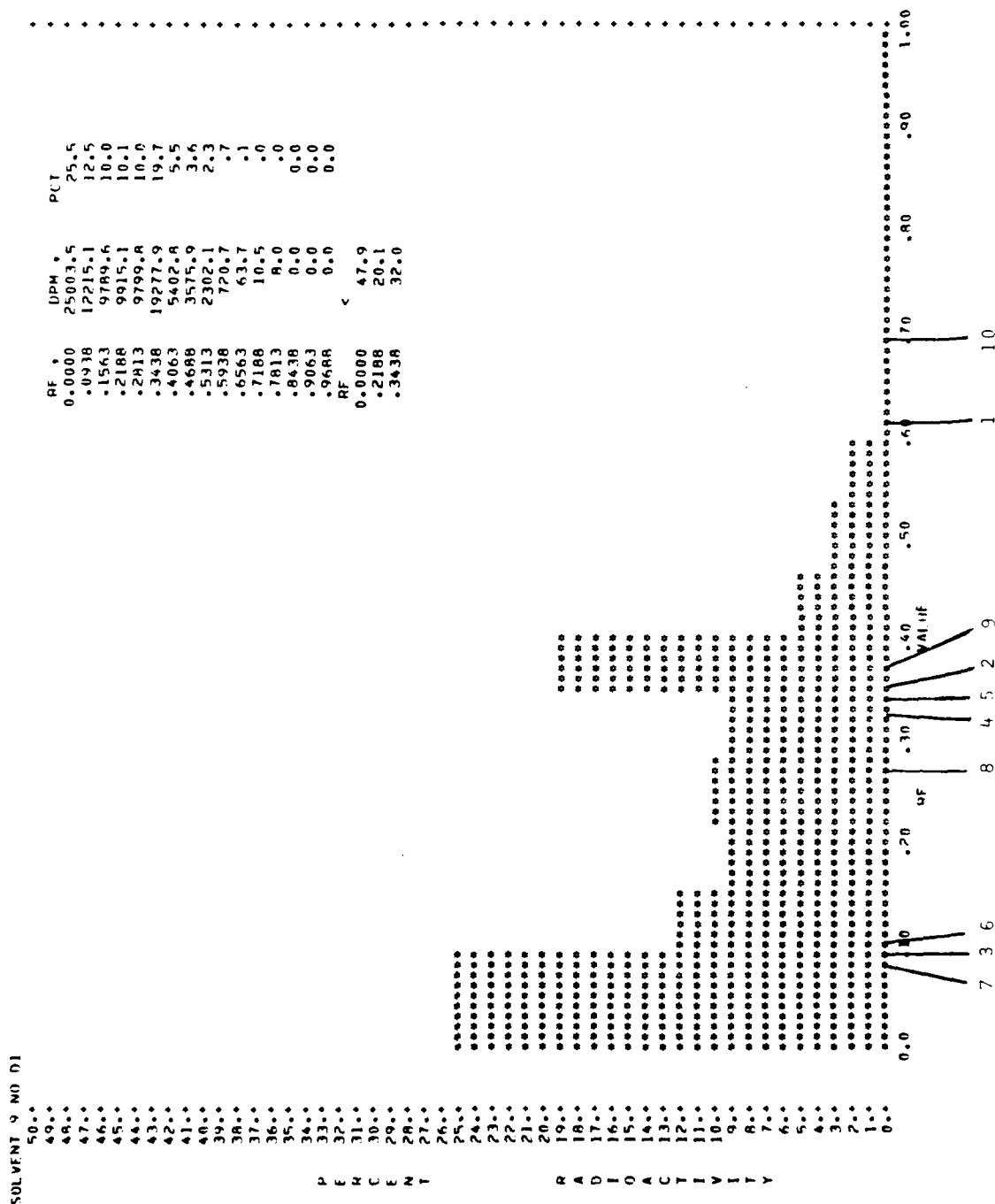


Figure 38-EI: Solvent IX

SOLVENT I NO D2

P	RF	DPM	PCT
50..	0.0000	2120.4	4.1
49..	.0438	1375.7	2.7
48..	.1563	1527.8	3.0
47..	.2188	1548.3	3.0
46..	.2813	1551.7	3.0
45..	.3438	1949.4	3.8
44..	.4063	7068.3	13.8
43..	.4688	8911.5	17.4
42..	.5313	3086.2	6.0
41..	.5938	3169.0	6.2
40..	.6563	4383.9	8.6
39..	.7188	11186.1	21.8
38..	.7813	3208.0	6.3
37..	.8438	98.4	.2
36..	.9063	10.4	.0
35..	.9688	3.4	.0
34..	RF		
33..	0.0000	6.8	
32..	.4688	50.1	
31..	.7188	43.1	
30..			
29..			
28..			
27..			
26..			
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24..			
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8..			
7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E R C E N T

R A D I O A C T I V I T Y

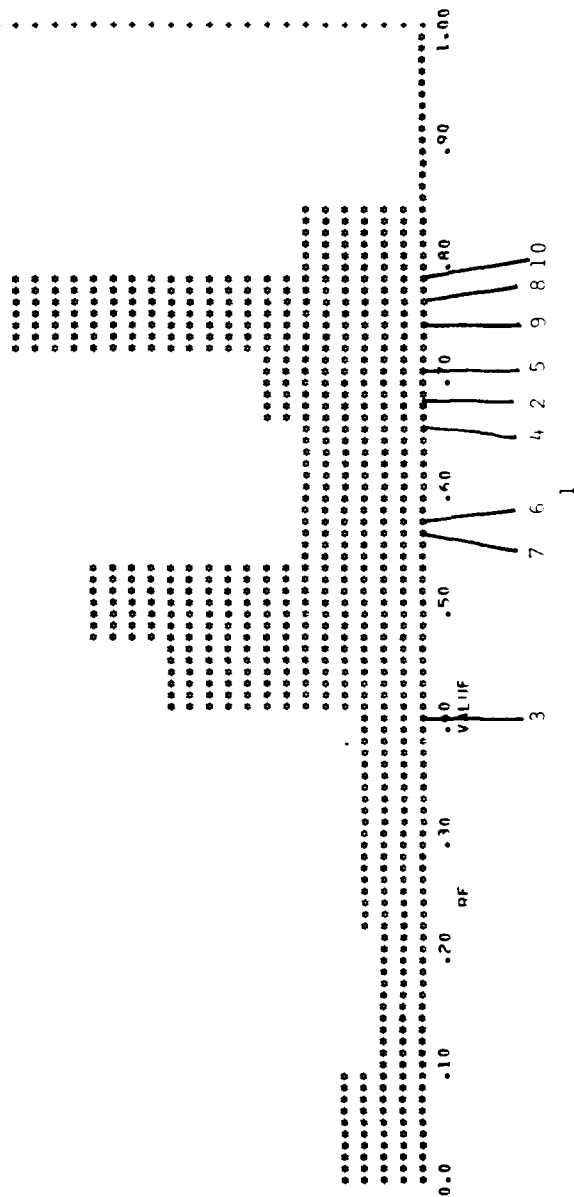


Figure 38-E2: Solvent I

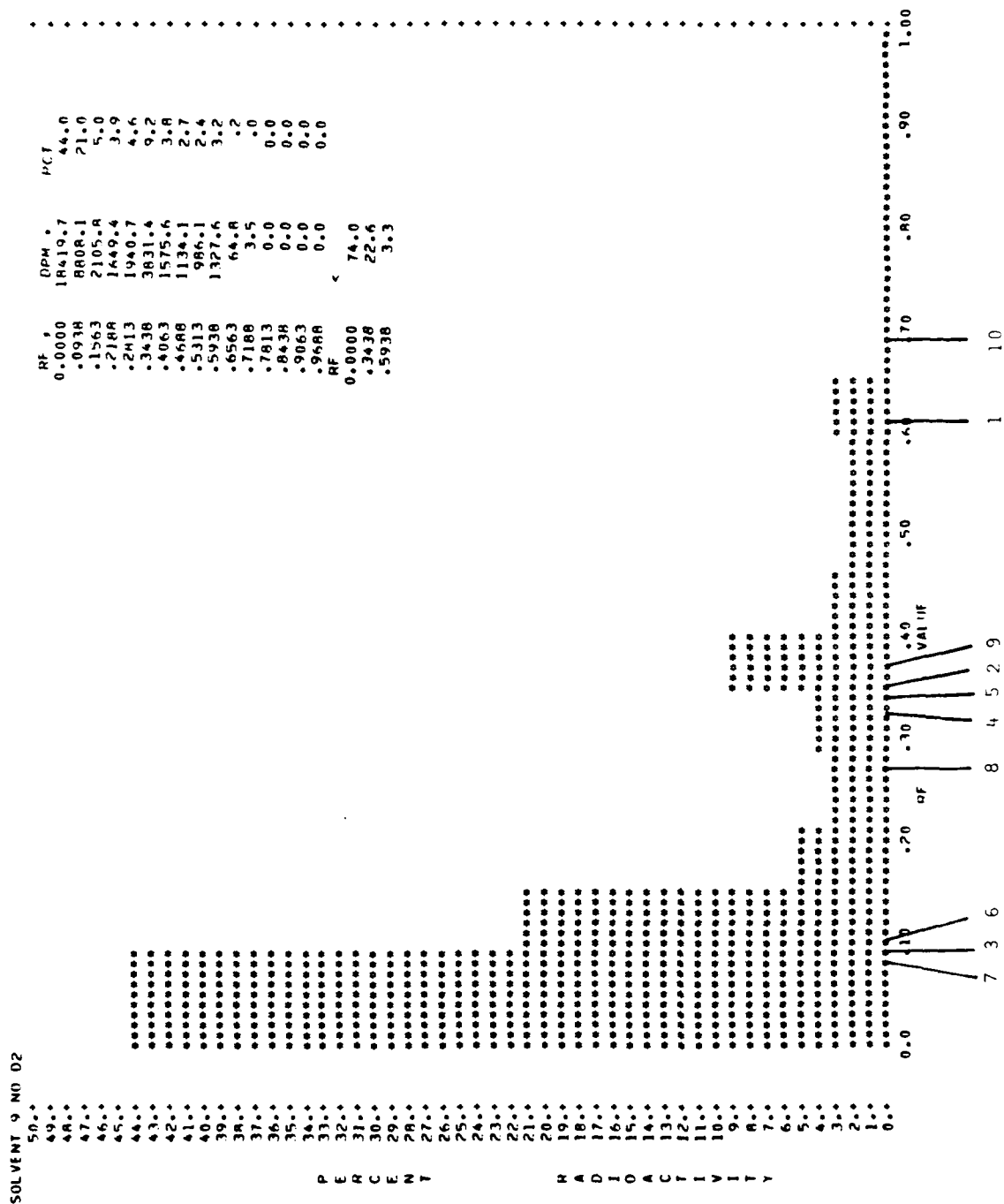


Figure 38-E2: Solvent IX

SOLVENT 1 NO 03

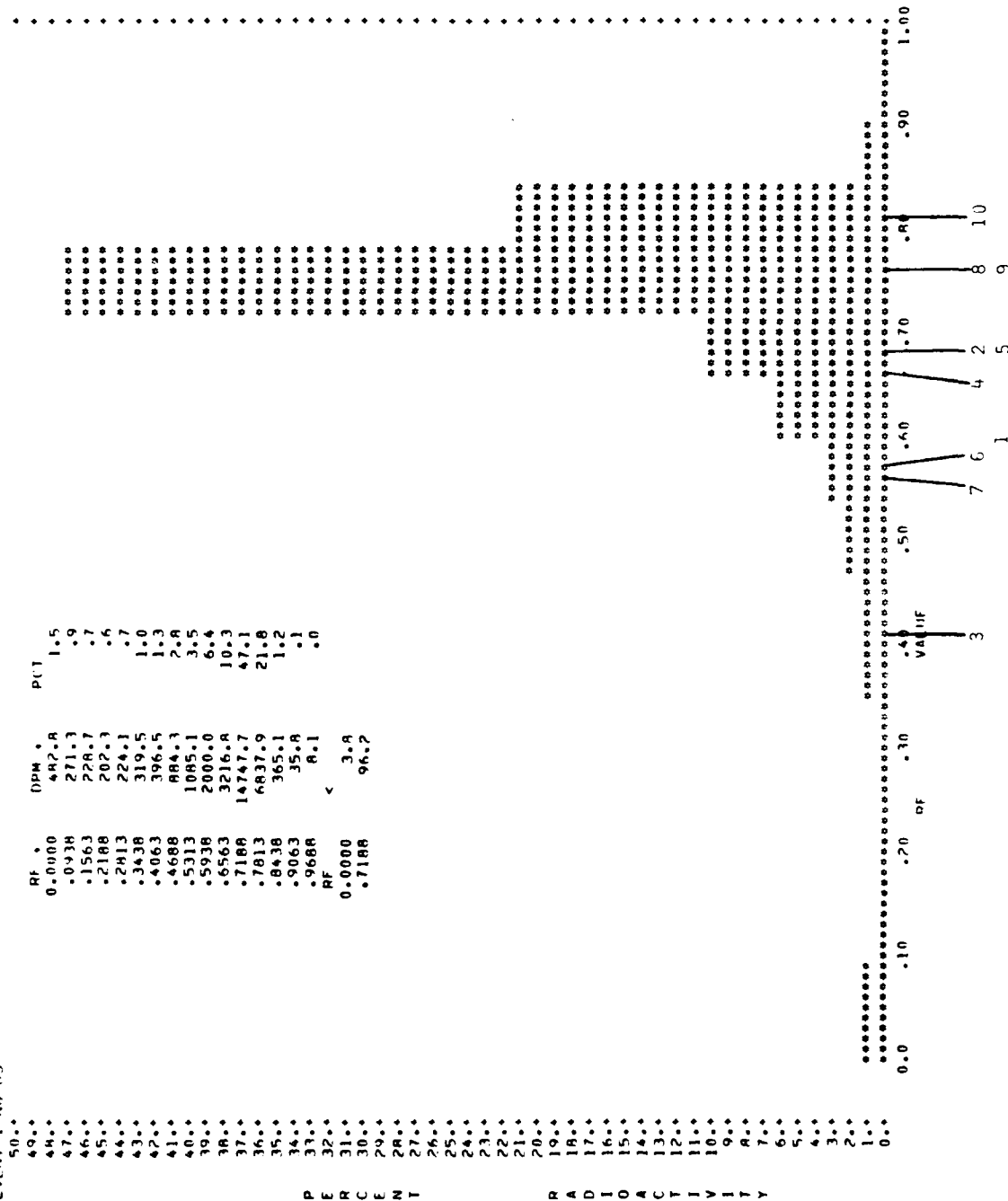
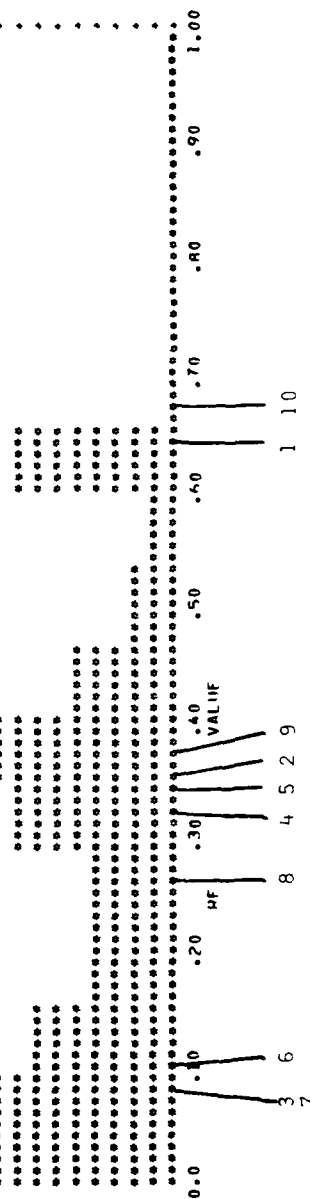


Figure 38-E3: Solvent I

RF	IPM	PCI
0.0000	4141.0	15.1
.0938	2100.6	7.7
.1563	1288.7	4.7
.2188	1273.1	4.6
.2813	2386.9	8.7
.3438	11144.9	40.6
.4063	1583.8	5.4
.4688	687.4	2.8
.5313	360.7	1.3
.5938	2421.3	8.8
.6563	84.4	.3
.7188	3.5	.0
.7813	0.0	0.0
.8438	0.0	0.0
.9063	0.0	0.0
.9688	0.0	0.0
RF	<	
0.0000	32.1	
.3438	58.8	
.5938	9.1	

Q W X U W Z -

RADIOACTIVITY

Figure 38-E₃: Solvent IX

SOLVENT 1 ND 04

RF	UPM	PCT
0.0000	1145.2	3.6
.0438	818.5	2.5
.1563	457.2	1.4
.2188	552.6	1.7
.2813	617.4	1.9
.3438	1430.6	4.4
.4063	5081.0	15.6
.4688	2913.3	8.9
.5313	1692.0	5.2
.5938	1748.0	5.4
.6563	3554.0	10.9
.7188	8047.5	24.7
.7813	4297.1	13.2
.8438	185.2	.6
.9063	31.0	.1
.9688	0.0	0.0
RF	<	
0.0000	7.5	
.4063	37.7	
.7188	54.8	

P F R C E N T

R A D I O C A T I V I T Y

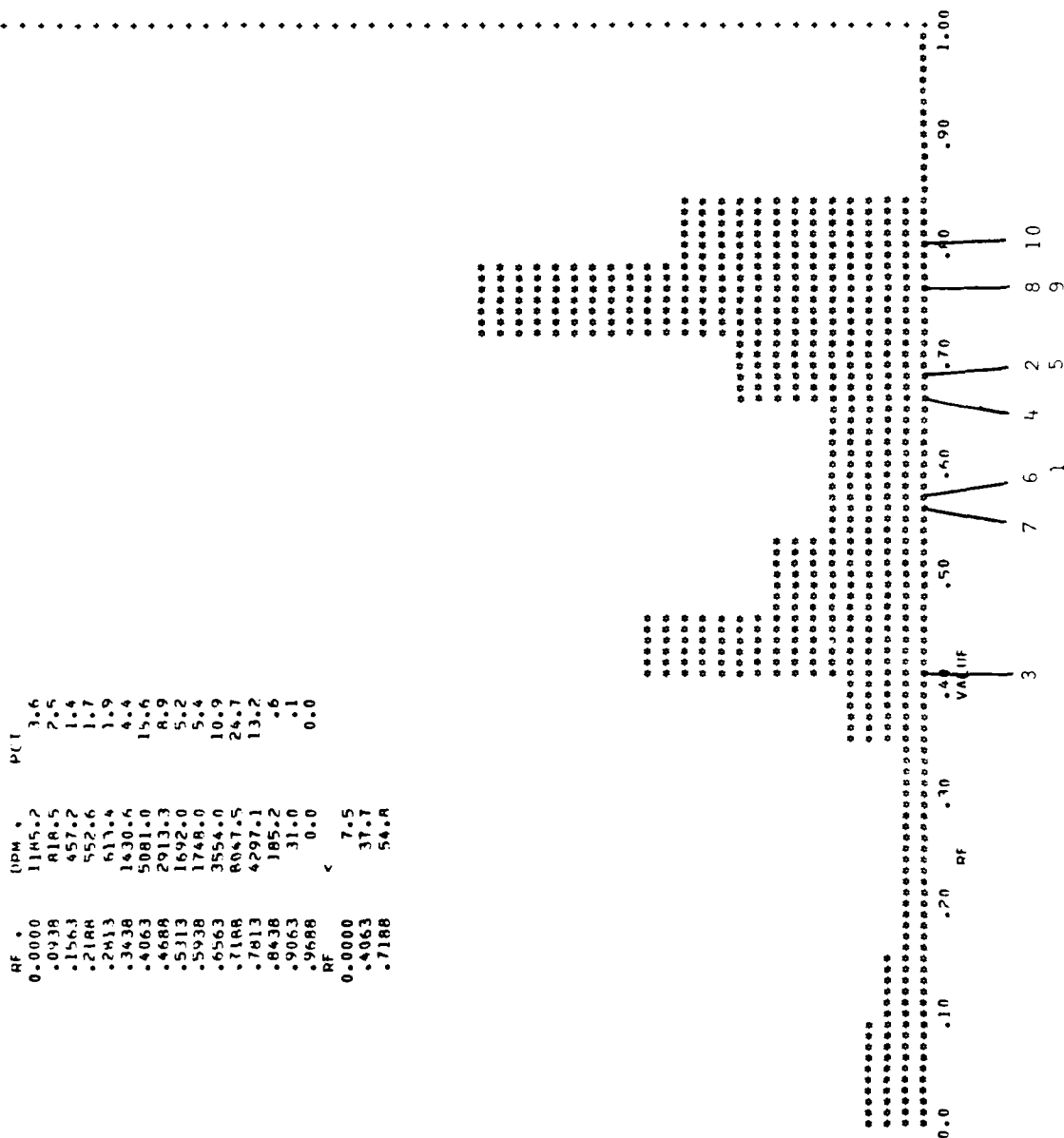


Figure 38-E4: Solvent I

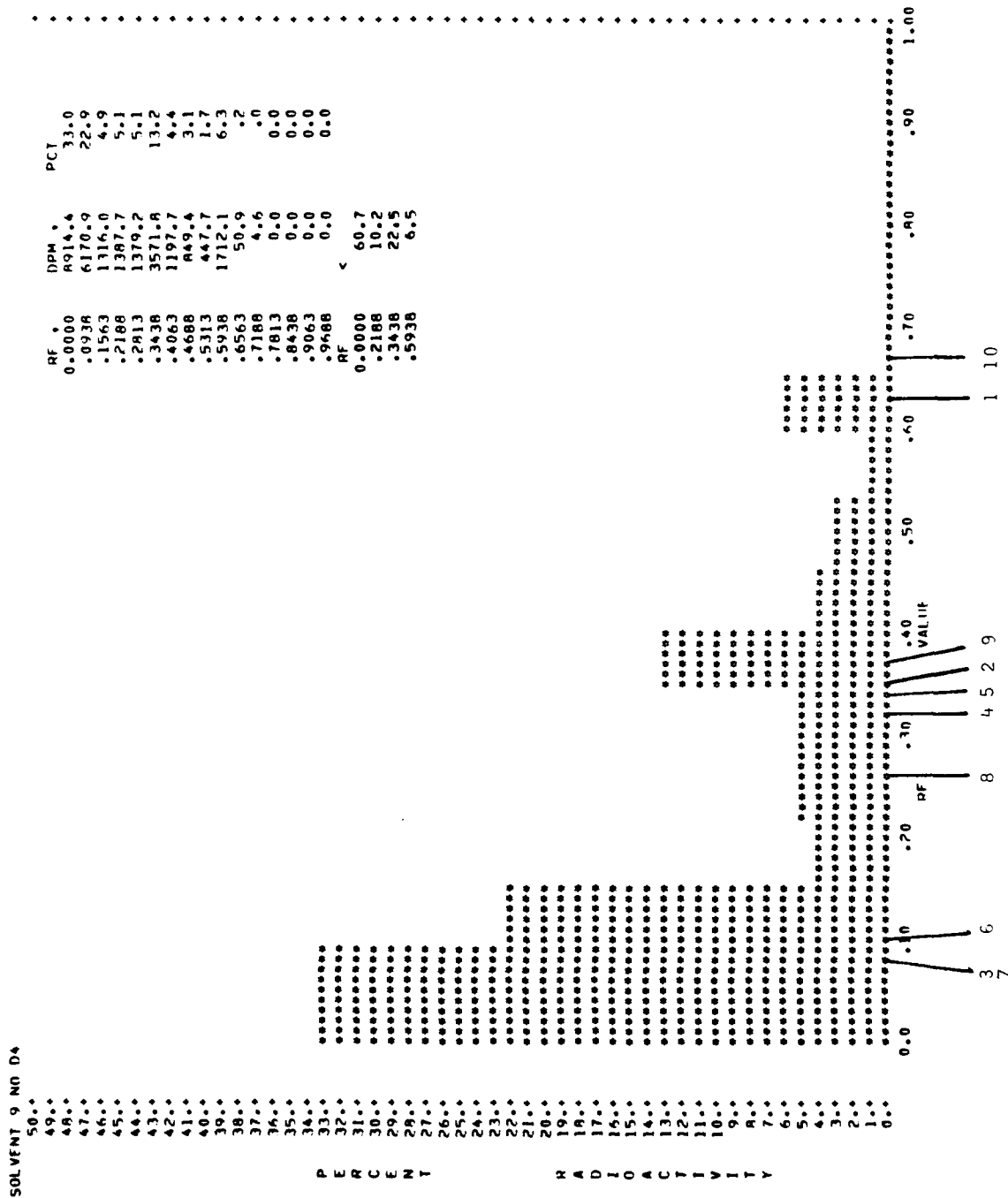


Figure 38-E4: Solvent IX

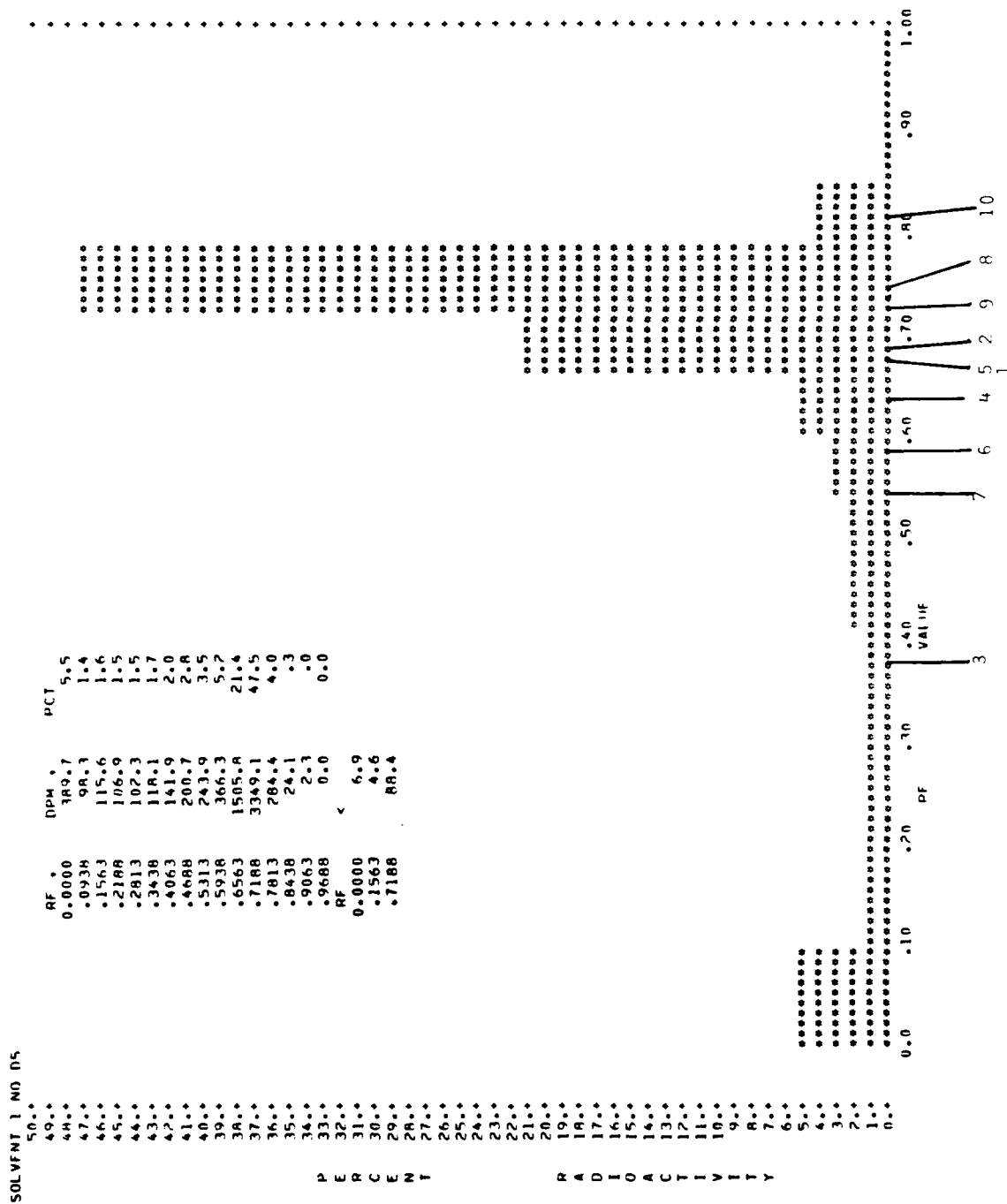


Figure 38-E5: Solvent I

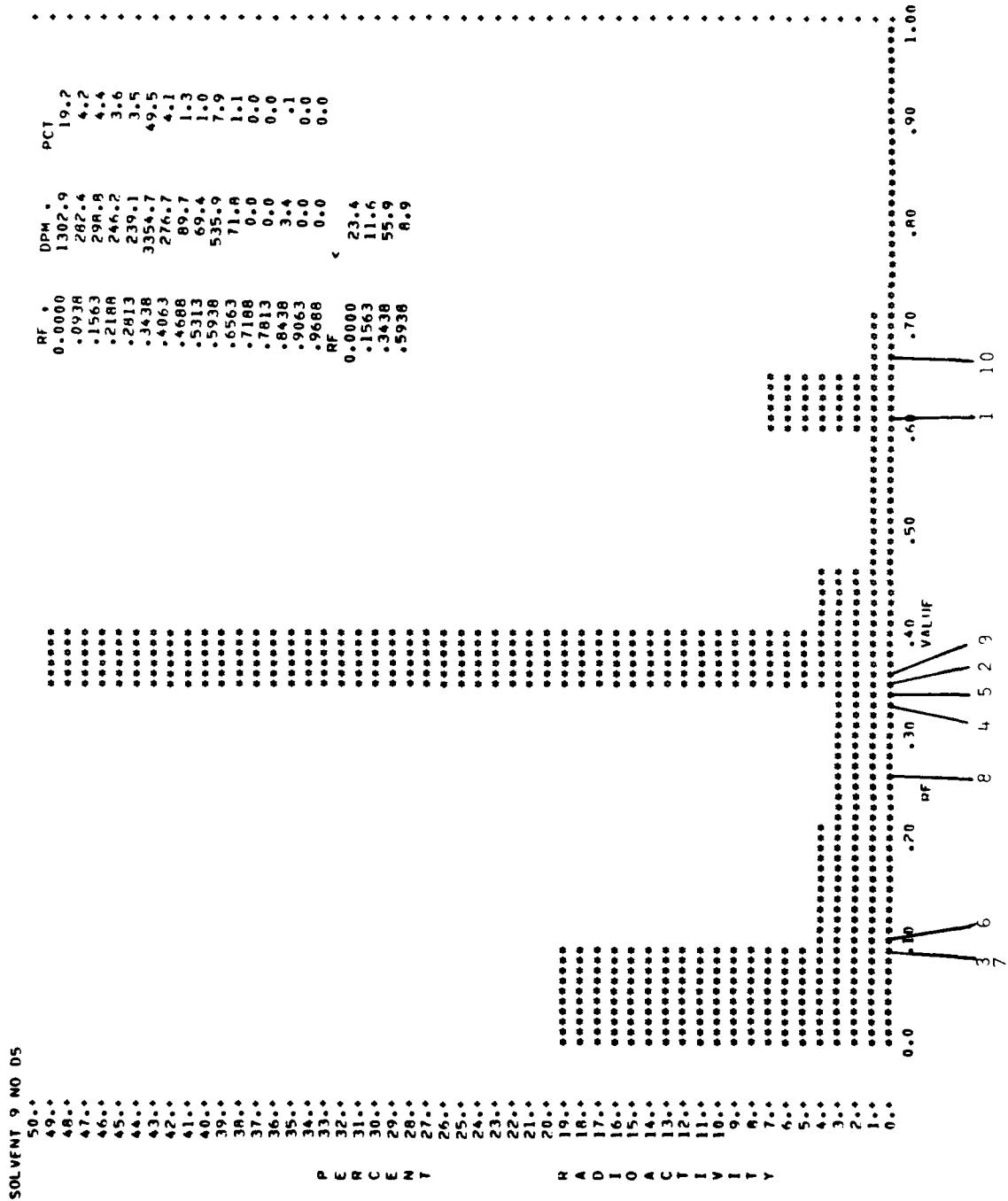
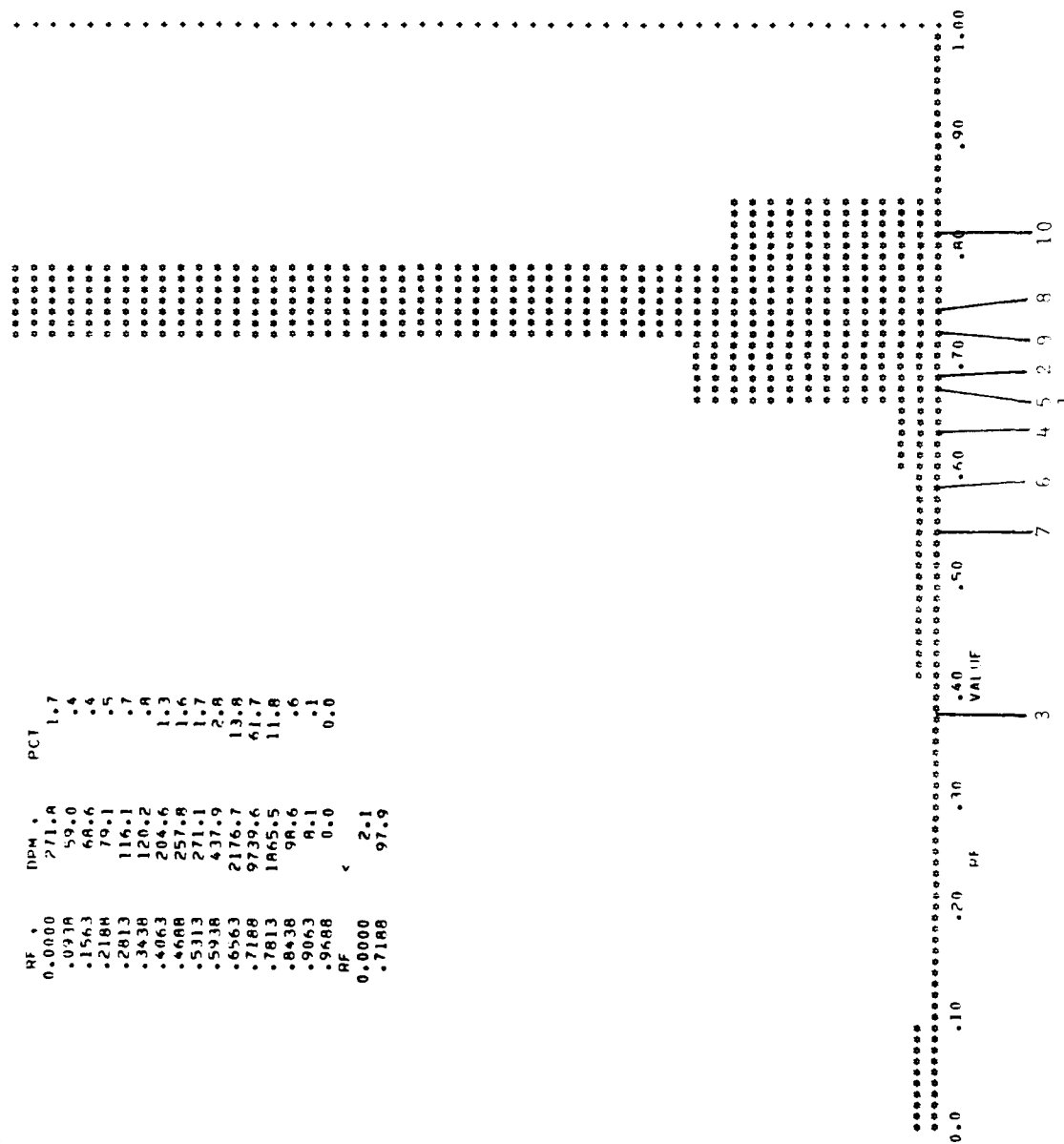


Figure 38-E5: Solvent IX

[illegible]

RF	DPM	PCT
0.0000	271.8	1.7
0.0318	59.0	4
1.563	68.6	5
2.184	79.1	5
2.813	116.1	7
3.348	120.2	8
4.063	204.6	1.3
4.668	257.8	1.6
5.313	271.1	1.7
5.938	437.9	2.8
6.563	2176.7	13.8
7.188	9739.6	61.7
7.813	1845.5	11.8
8.438	98.6	6
9.063	8.1	0.1
9.688	0.0	0.0
RF	<	
0.0000	2.1	
7.188	97.9	

Figure 38-E₆: Solvent I

SOLVENT 9 NO D6

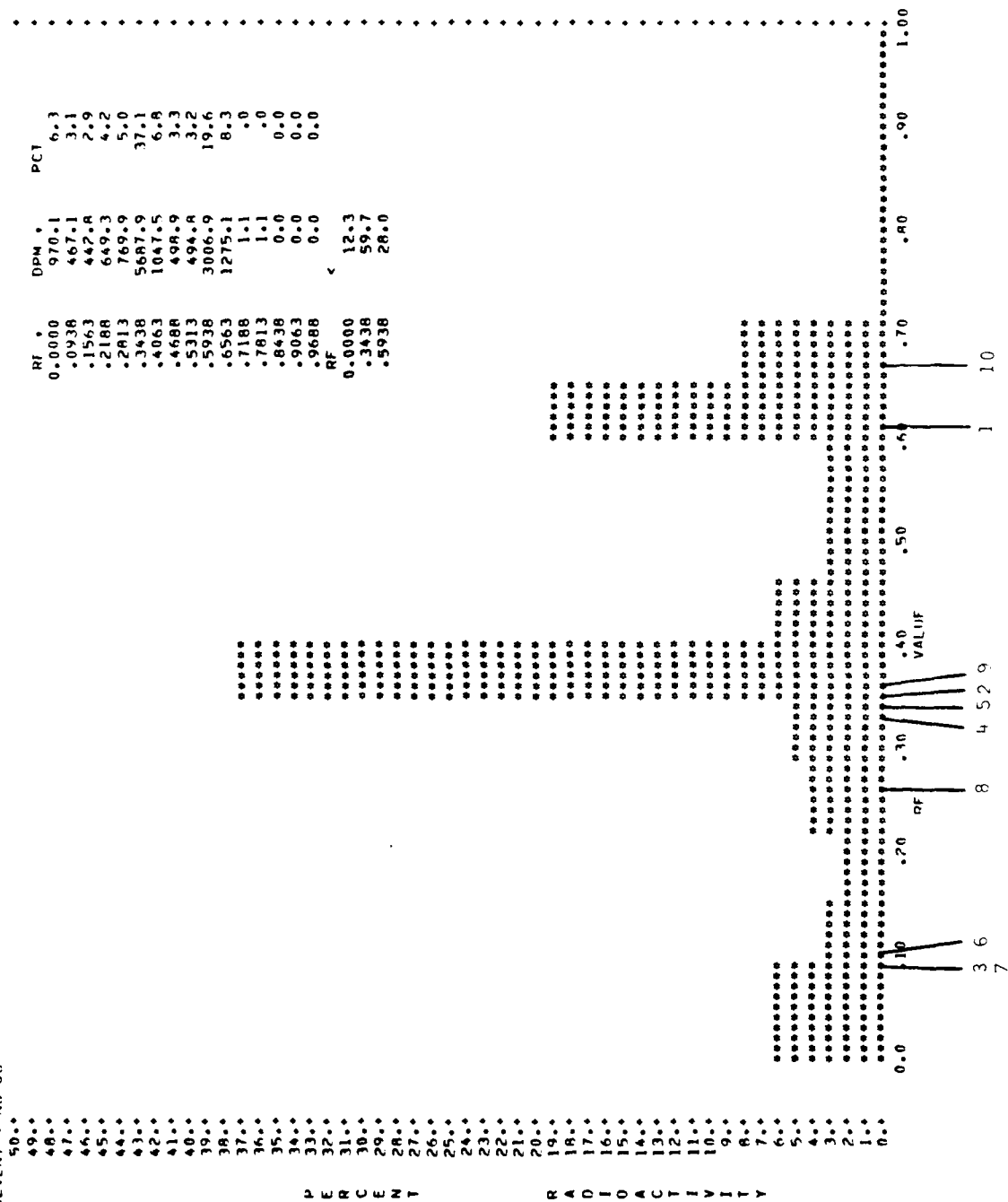


Figure 38-E6: Solvent IX

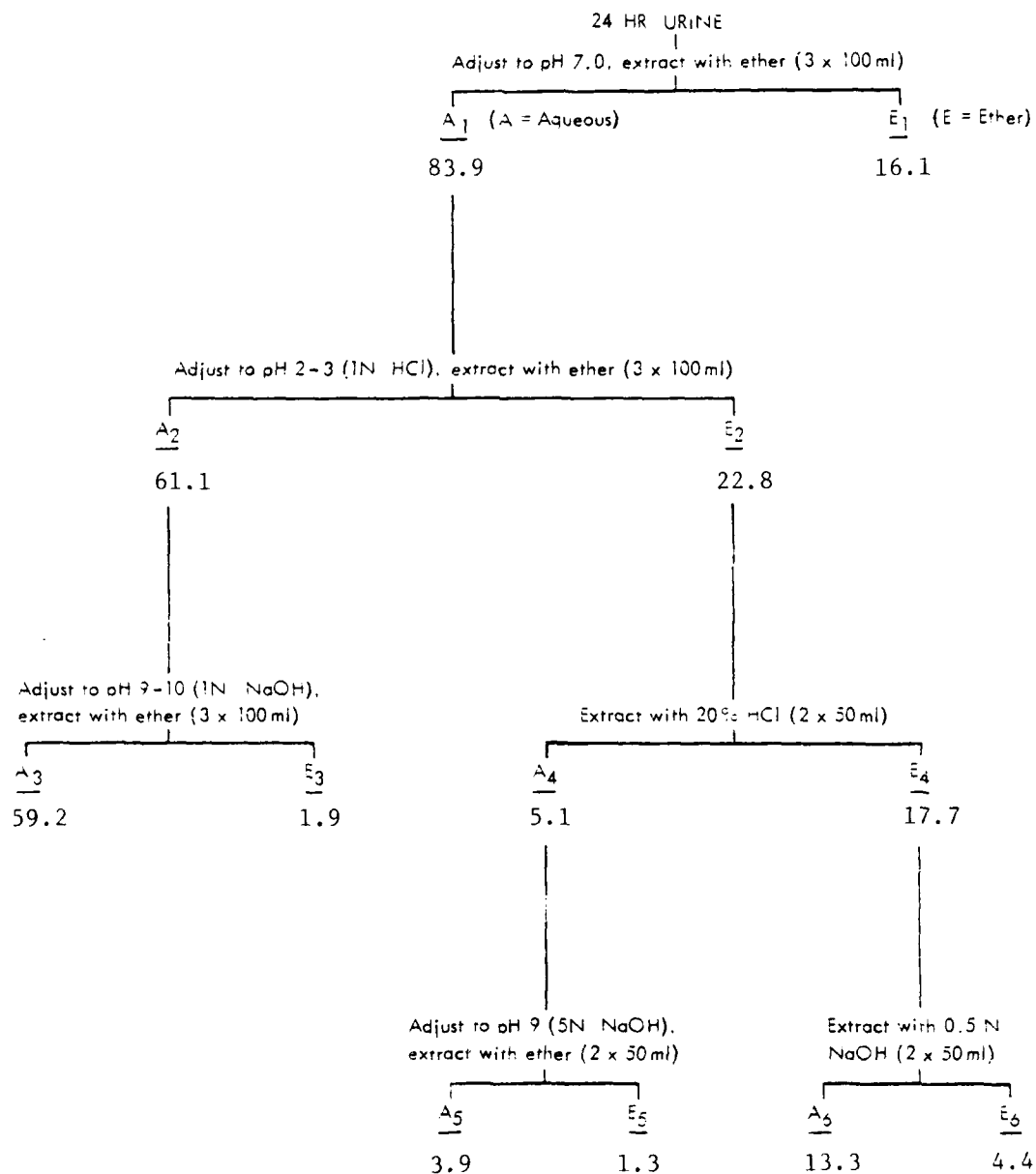
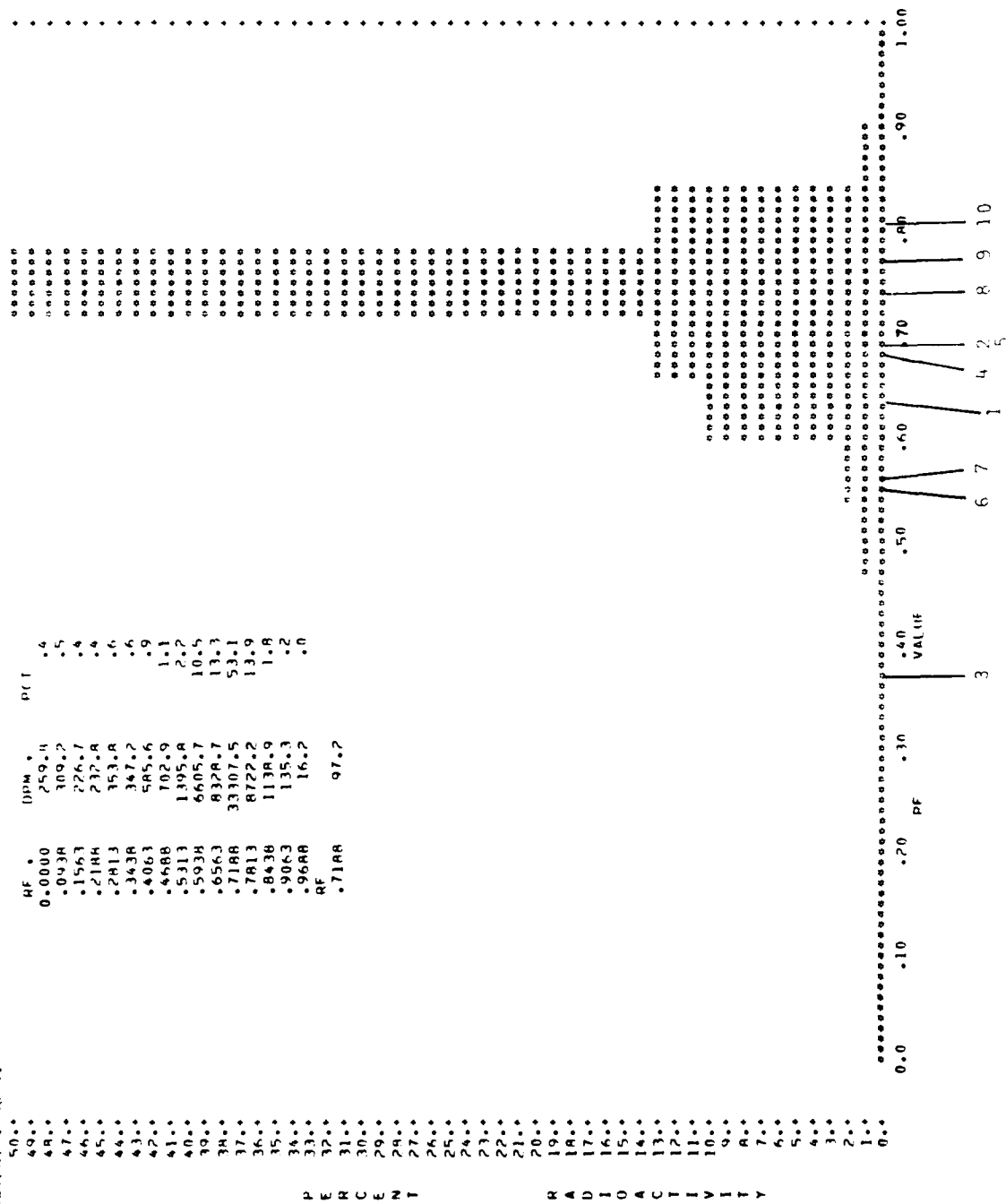


Figure 39: Fractionation of 24-Hr Urine from Dogs Treated Dermal with ¹⁴C-TNT. Values indicate the percentage of extractable radioactivity in each fraction.

Figure 40, E₁-E₆: TLC of Ether-Extractable Products Obtained from 24-Hr Urine of Dogs Treated Dermally with ¹⁴C-TNT. Extractions were performed at different pH conditions according to the scheme described in the preceding figure. Solvent I, n-butanol:acetic acid:water, 10:1:1; Solvent IX, toluene:acetic acid, 4:1. For reference metabolites (1-10) see Figure 26 or Table 19. Reference metabolites are:

- | | |
|-------------------------------|--|
| 1. Trinitrotoluene (TNT) | 6. 4,6-Diamino-2-nitrotoluene |
| 2. Trinitrobenzyl alcohol | 7. 2,6-Diamino-4-nitrotoluene |
| 3. Trinitrobenzoic Acid | 8. 4-Hydroxylamino-2,6-dinitrotoluene |
| 4. 4-Amino-2,6-dinitrotoluene | 9. 2-Hydroxylamino-4,6-dinitrotoluene |
| 5. 2-Amino-4,6-dinitrotoluene | 10. 2,6,2',6'-Tetranitro-4,4'-azoxytoluene |

Figure 40 follows

Figure 40-E₁: Solvent I

SOLVENT 9 NO DI

50.0	0.0000	13430.1	20.7
49.0	.0434	3996.5	6.2
48.0	.1563	3274.0	5.0
47.0	.2148	2737.6	4.2
46.0	.2413	3968.8	6.1
45.0	.3438	9434.0	14.5
44.0	.4063	4327.5	6.7
43.0	.4688	6384.3	9.8
42.0	.5313	5198.8	8.0
41.0	.5938	9965.3	15.4
40.0	.6563	2067.1	3.2
39.0	.7188	63.0	.1
38.0	.7813	3.5	.0
37.0	.8438	2.3	.0
36.0	.9063	0.0	0.0
35.0	.9688	0.0	0.0
34.0	RF		
33.0	0.0000	36.1	
32.0	.3438	27.3	
31.0	.4688	17.9	
30.0	.5938	18.7	

P E R C E N T

R A D I O A C T I V I T Y

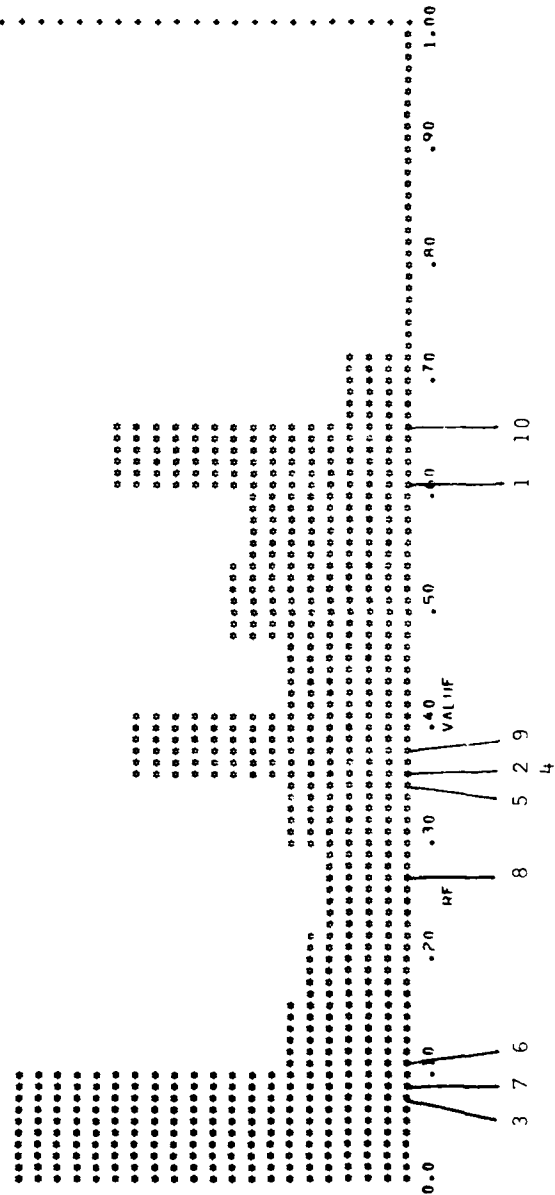


Figure 40-E1: Solvent IX

SOLVENT 1 NO D2

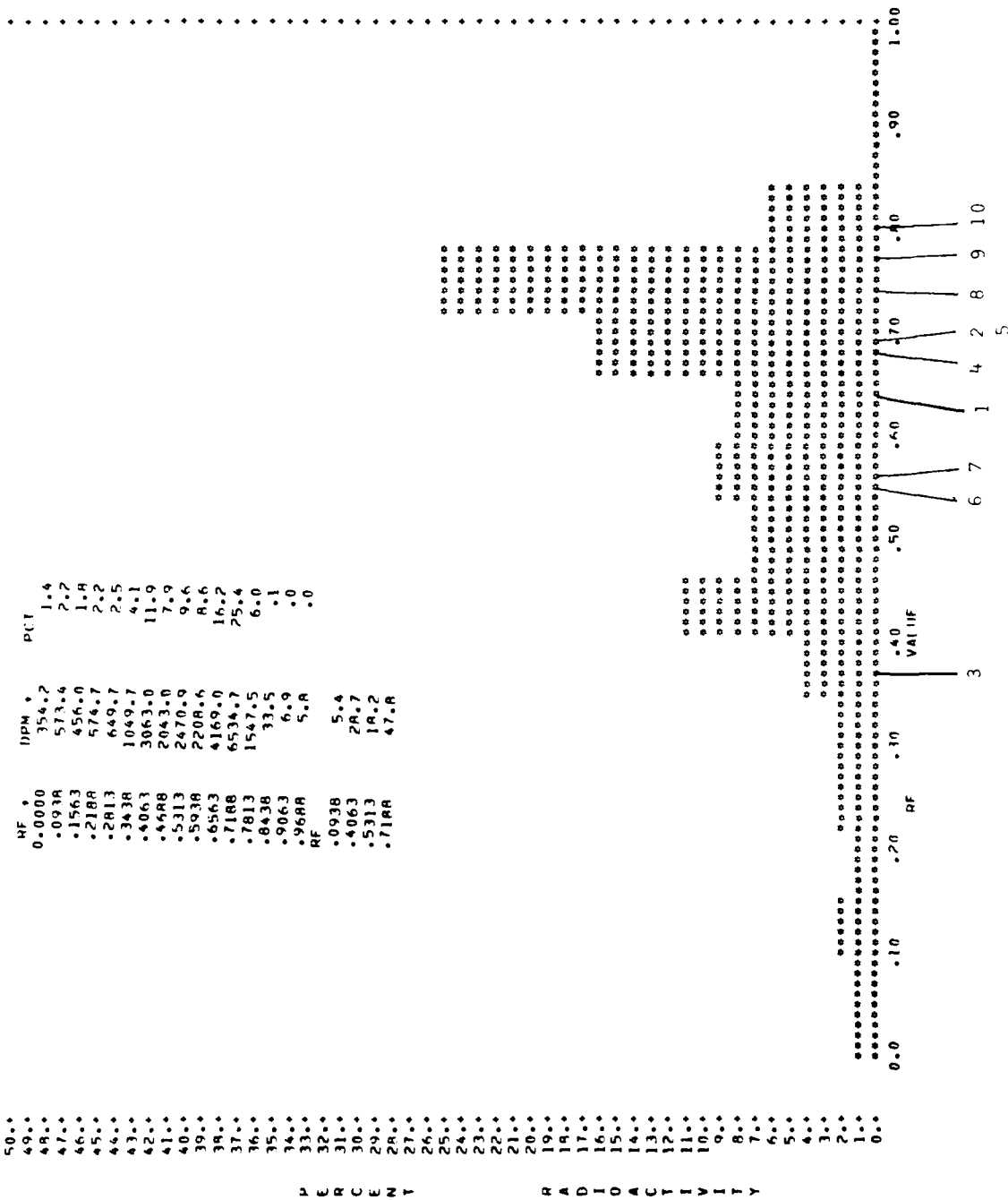


Figure 40-E2: Solvent I

SOLVENT 9 NO D2

50.0	RF	0.0000	9480.9	PPM	34.5
49.0	PE	.0938	7198.8		26.2
48.0	R	.1563	1926.7		7.0
47.0	R	.2188	994.3		3.6
46.0	C	.2813	1190.1		4.4
45.0	E	.3438	1417.3		5.2
44.0	N	.4063	1090.6		4.0
43.0	T	.4688	2366.6		4.6
42.0		.5313	1309.0		4.8
41.0		.5938	391.9		1.4
40.0		.6563	127.3		.5
39.0		.7188	8.0		.0
38.0		.7813	0.0		0.0
37.0		.8438	0.0		0.0
36.0		.9063	0.0		0.0
35.0		.9688	0.0		0.0
34.0		RF	0.0000		71.2
33.0					13.5
32.0					15.3
31.0					
30.0					
29.0					
28.0					
27.0					
26.0					
25.0					
24.0					
23.0					
22.0					
21.0					
20.0					
19.0					
18.0					
17.0					
16.0					
15.0					
14.0					
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12.0					
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10.0					
9.0					
8.0					
7.0					
6.0					
5.0					
4.0					
3.0					
2.0					
1.0					
0.0					

P E R C E N T

R A D I O A C T I V I T Y

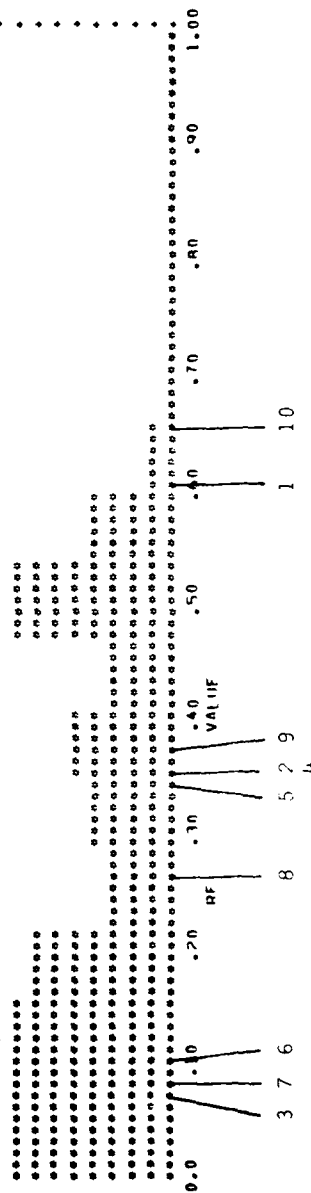


Figure 40-E2: Solvent IX

SOLVENT 1 NO 03

RF	DPM	RET
0.0000	222.0	4.4
.0418	117.9	2.3
.1563	90.6	1.8
.2188	75.1	1.5
.2813	81.6	1.6
.3438	116.3	2.3
.4063	99.8	2.0
.4688	134.1	2.7
.5313	225.6	4.5
.5938	986.1	17.9
.6563	994.1	19.7
.7188	1526.4	30.2
.7813	369.0	7.3
.8438	75.6	1.5
.9063	22.0	.4
.9688	0.0	0.0
RF		
0.0000	9.9	
.3438	5.9	
.7188	84.0	

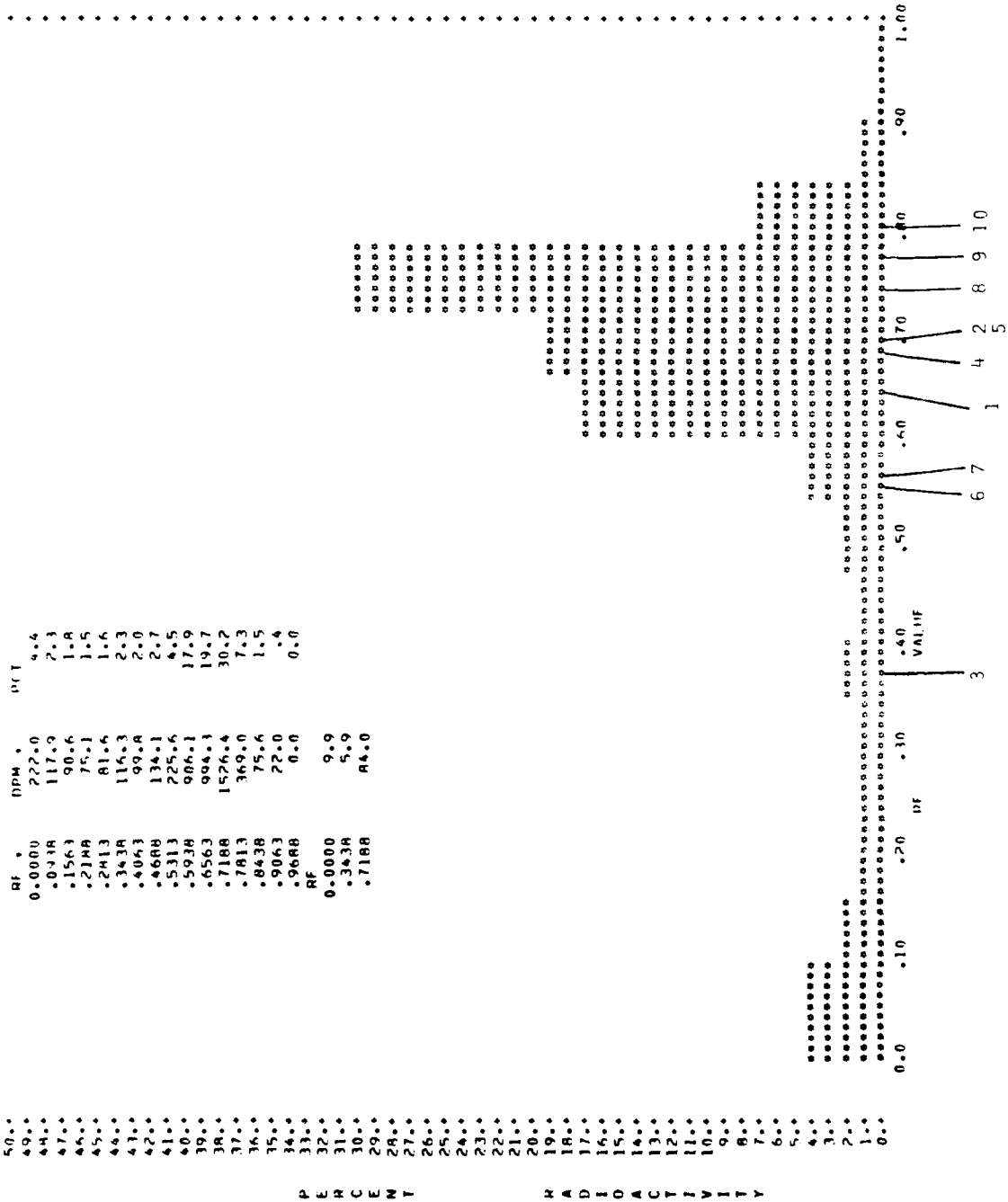


Figure 40-E3: Solvent I

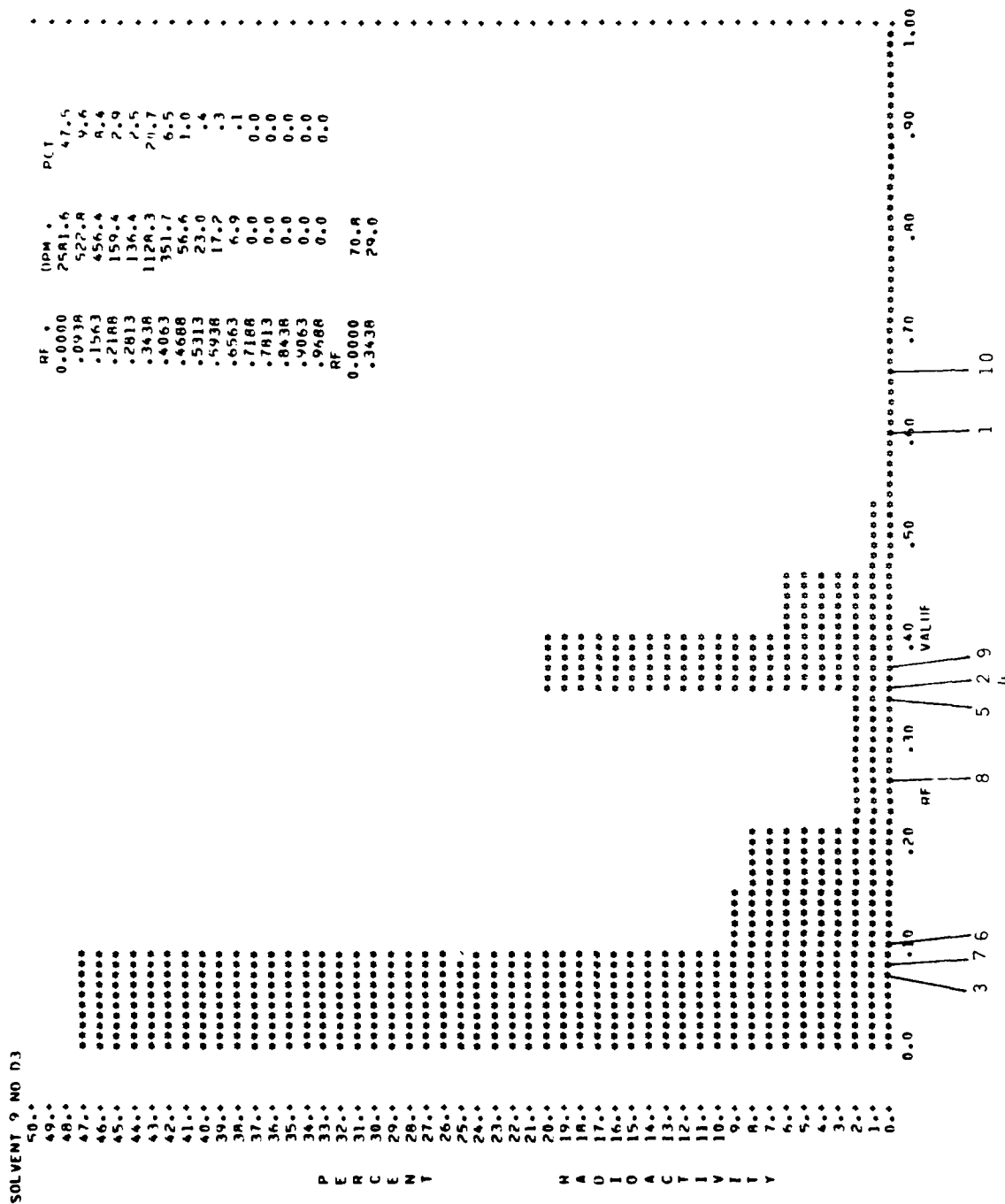


Figure 40-E3: Solvent IX

SOLVENT 1 NO D4

50.0	RF	UPM	PCT
49.0	0.0000	266.7	1.6
48.0	.0938	286.2	1.7
47.0	.1563	299.8	1.7
46.0	.2188	266.3	1.5
45.0	.2813	250.9	1.5
44.0	.3438	523.1	3.0
43.0	.4063	2422.5	14.1
42.0	.4688	889.0	5.2
41.0	.5313	647.1	3.8
40.0	.5938	772.1	4.5
39.0	.6563	1375.7	8.0
38.0	.7188	5357.2	31.2
37.0	.7813	3527.9	20.5
36.0	.8438	220.8	1.3
35.0	.9063	67.4	.4
34.0	.9688	16.3	.1
33.0	RF		
32.0	.1563	8.0	
31.0	.4063	26.1	
30.0	.7188	65.9	

P E R C E N T

H A D I O A C T I V I T Y

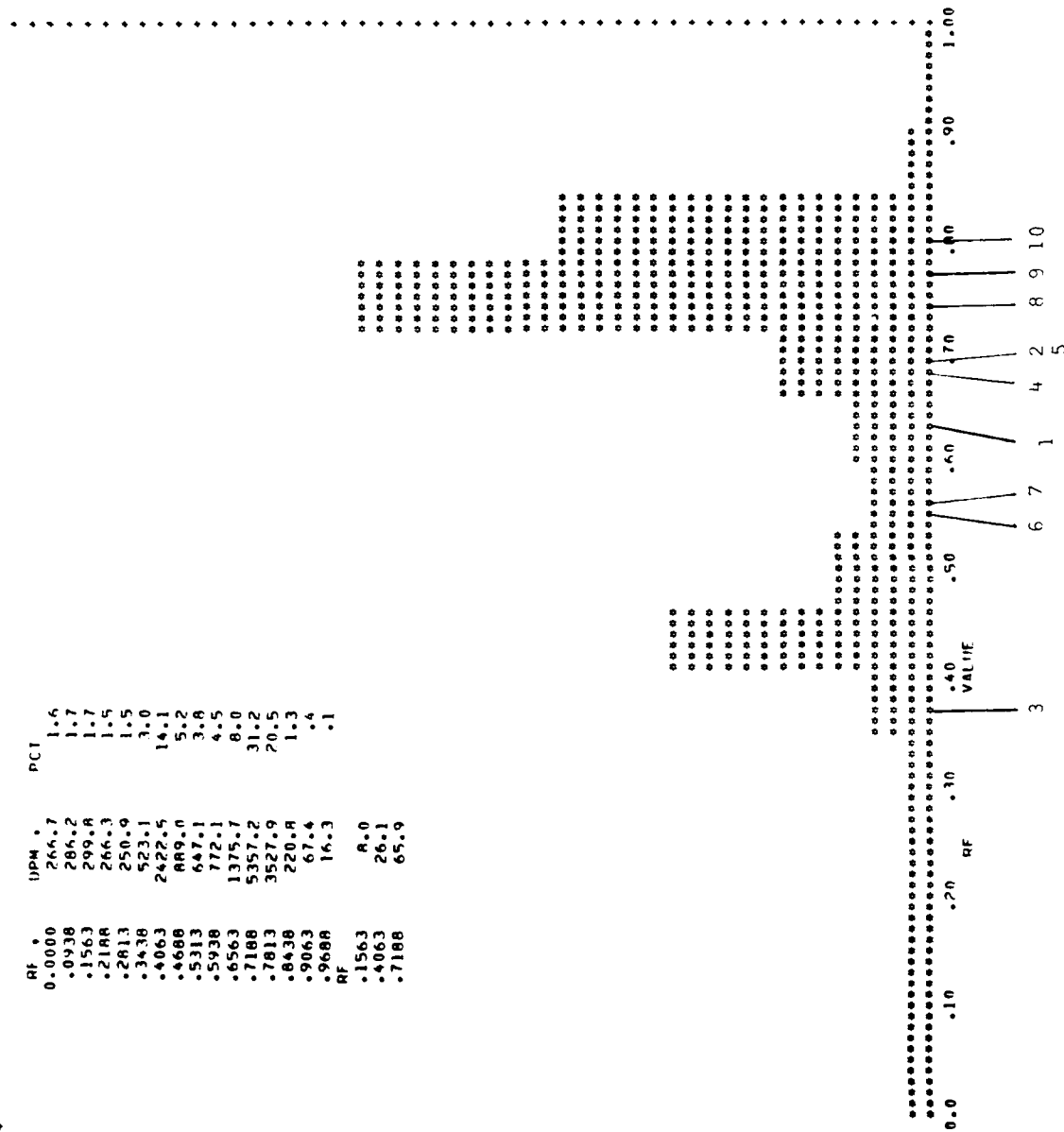


Figure 40-E₄: Solvent I

SOLVENT 9 NO DA

50..	RF	DPM	PCT
49..	0.0000	4559.5	27.1
48..	.0938	3465.7	20.6
47..	.1563	950.6	5.7
46..	.2188	609.2	3.6
45..	.2813	1135.6	6.8
44..	.3438	1257.8	7.5
43..	.4063	685.5	4.1
42..	.4688	1903.4	11.3
41..	.5313	815.0	4.9
40..	.5938	1068.2	6.4
39..	.6563	341.9	2.0
38..	.7188	5.8	0.0
37..	.7813	0.0	0.0
36..	.8438	0.0	0.0
35..	.9063	0.0	0.0
34..	.9688	0.0	0.0
33..	RF		
32..	0.0000	57.0	
31..	.3438	18.3	
30..	.4688	16.2	
29..	.5938	8.4	
28..			
27..			
26..			
25..			
24..			
23..			
22..			
21..			
20..			
19..			
18..			
17..			
16..			
15..			
14..			
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12..			
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7..			
6..			
5..			
4..			
3..			
2..			
1..			
0..			

P E R C E N T

R A D I O A C T I V I T Y

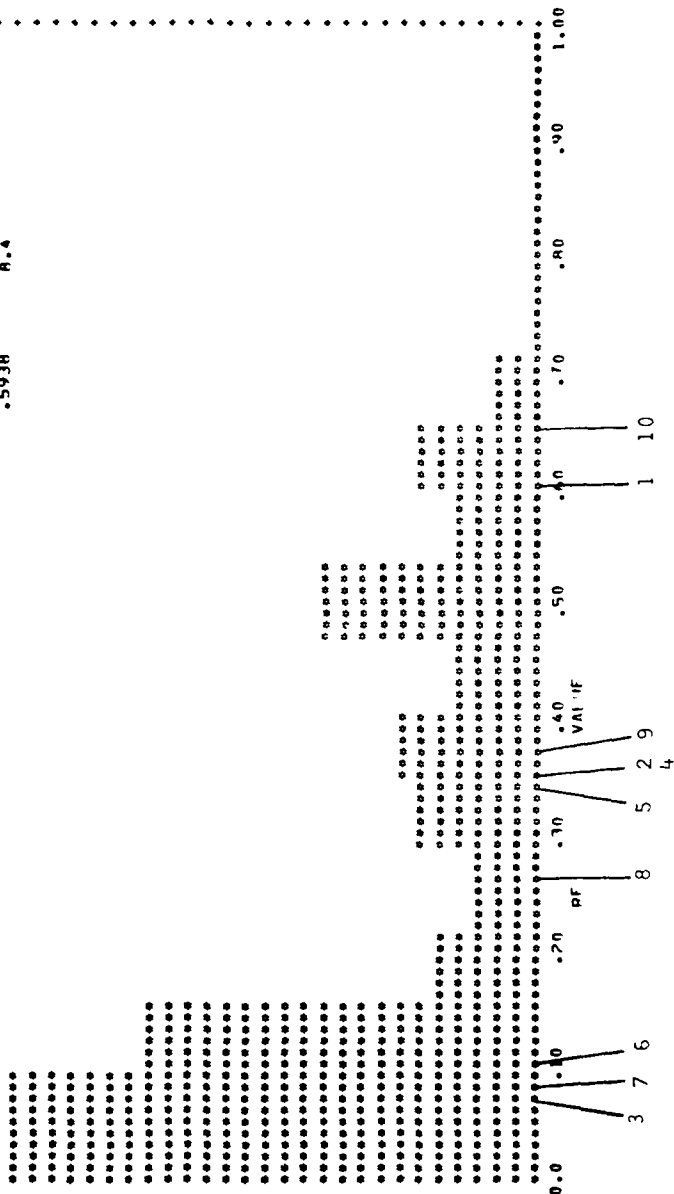


Figure 40-E4: Solvent IX

SOLVENT 1 NO 15

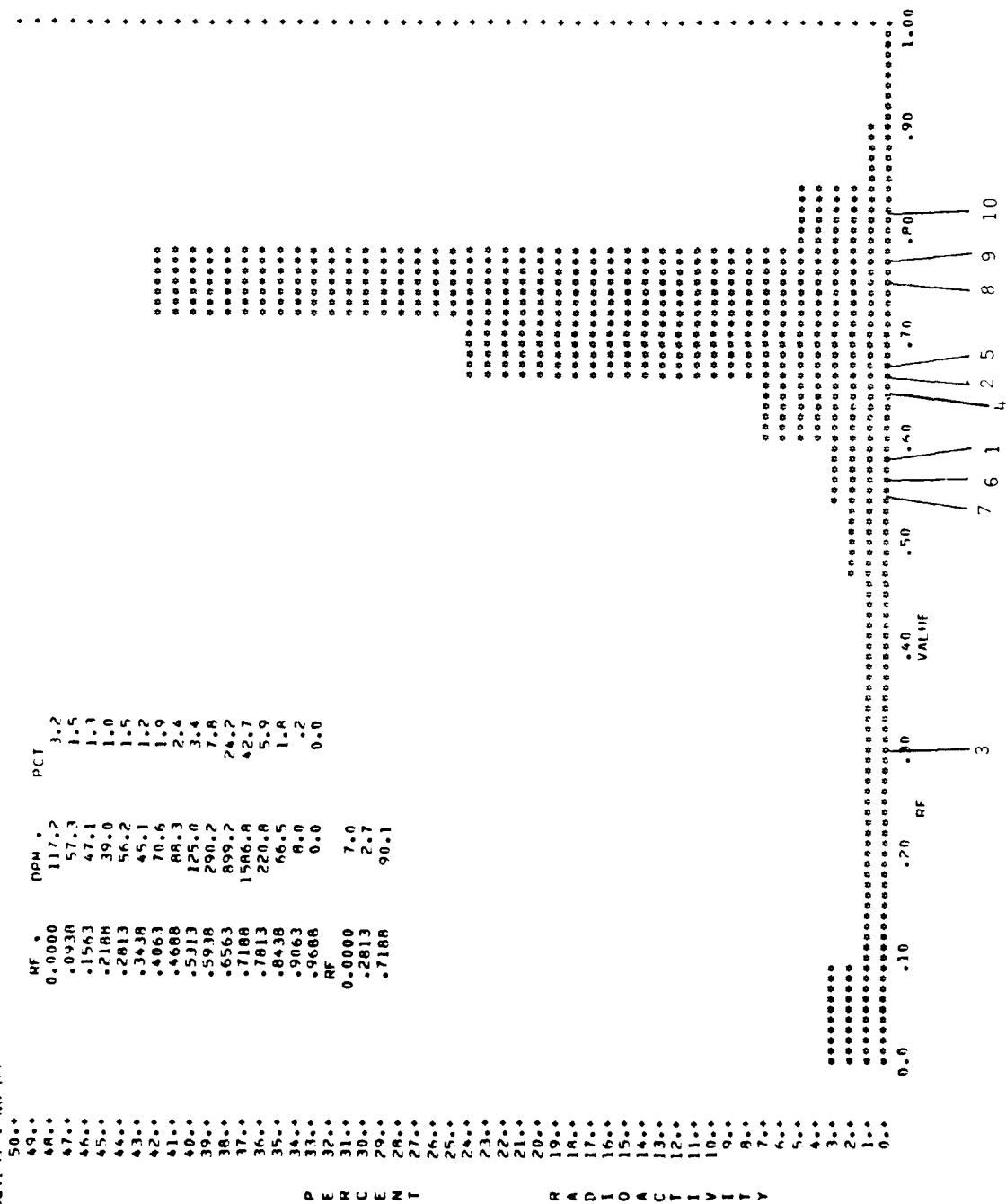


Figure 40-E5: Solvent I

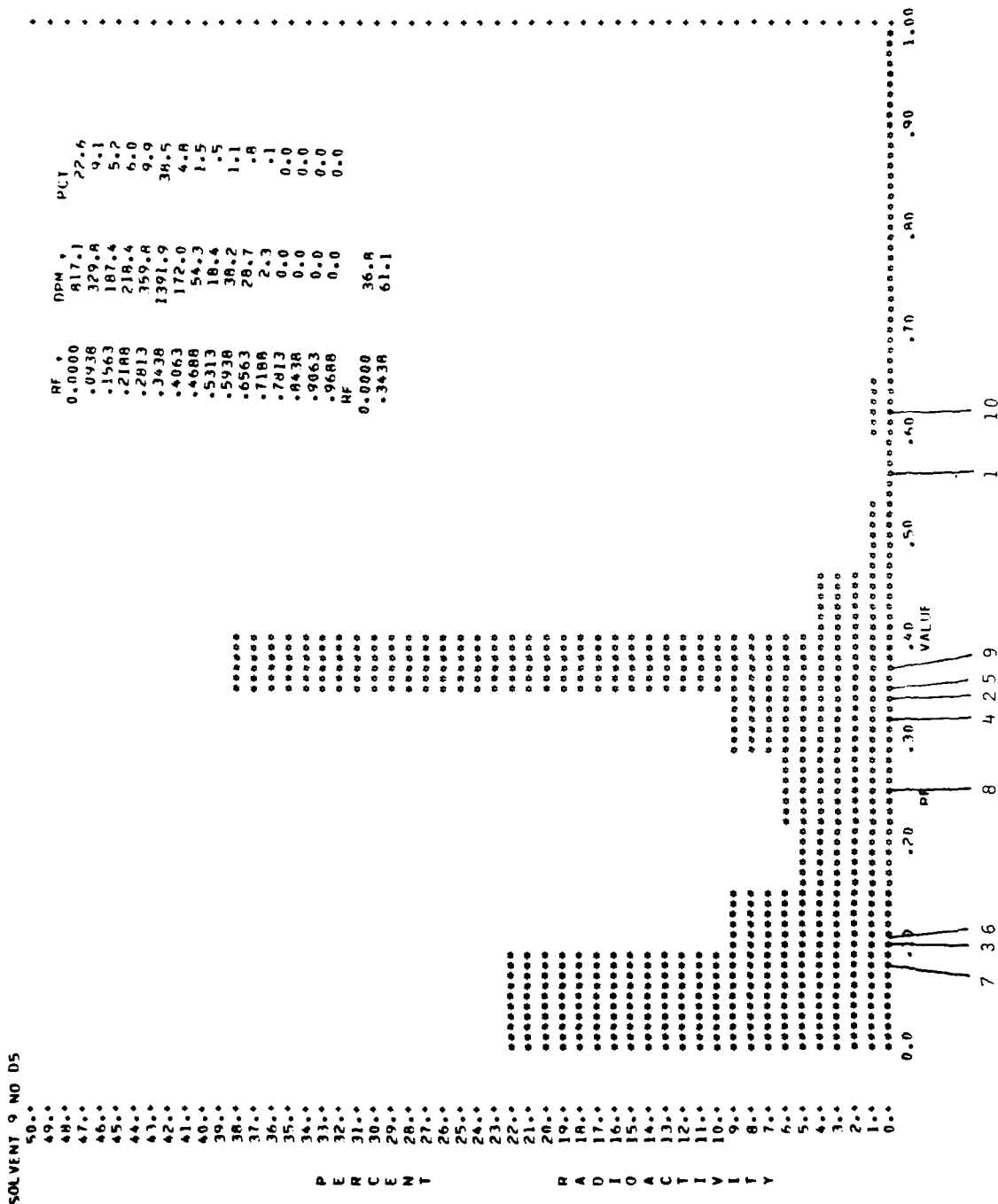


Figure 40-E5: Solvent IX

SOLVENT 1 NO D6

RF	DPM	PCT
0.0000	201.8	1.6
.49.0	50.9	.4
.023H	47.4	.4
.156J	56.3	.4
.218H	67.7	.5
.281J	102.3	.8
.343H	134.0	1.1
.406J	149.1	1.2
.468H	197.7	1.6
.531J	366.5	2.9
.593H	1654.8	13.2
.656J	7782.6	62.1
.718H	1341.7	10.7
.781J	311.0	2.5
.843H	65.1	.5
.906J	0.0	0.0
.968H		
RF		
0.0000	2.4	
.718H	97.6	

P E R R C E N T

R A D I O A C T I V I T Y

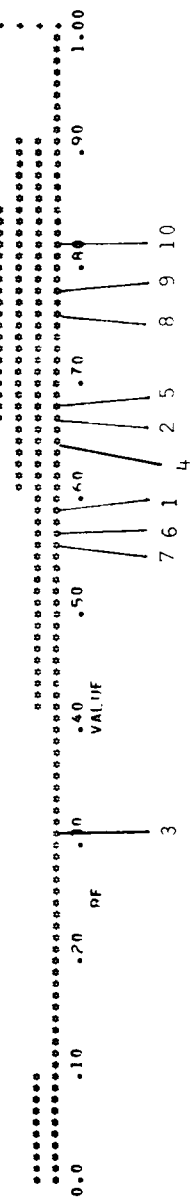


Figure 40-E₆: Solvent I

SOLVENT 4 NO 06

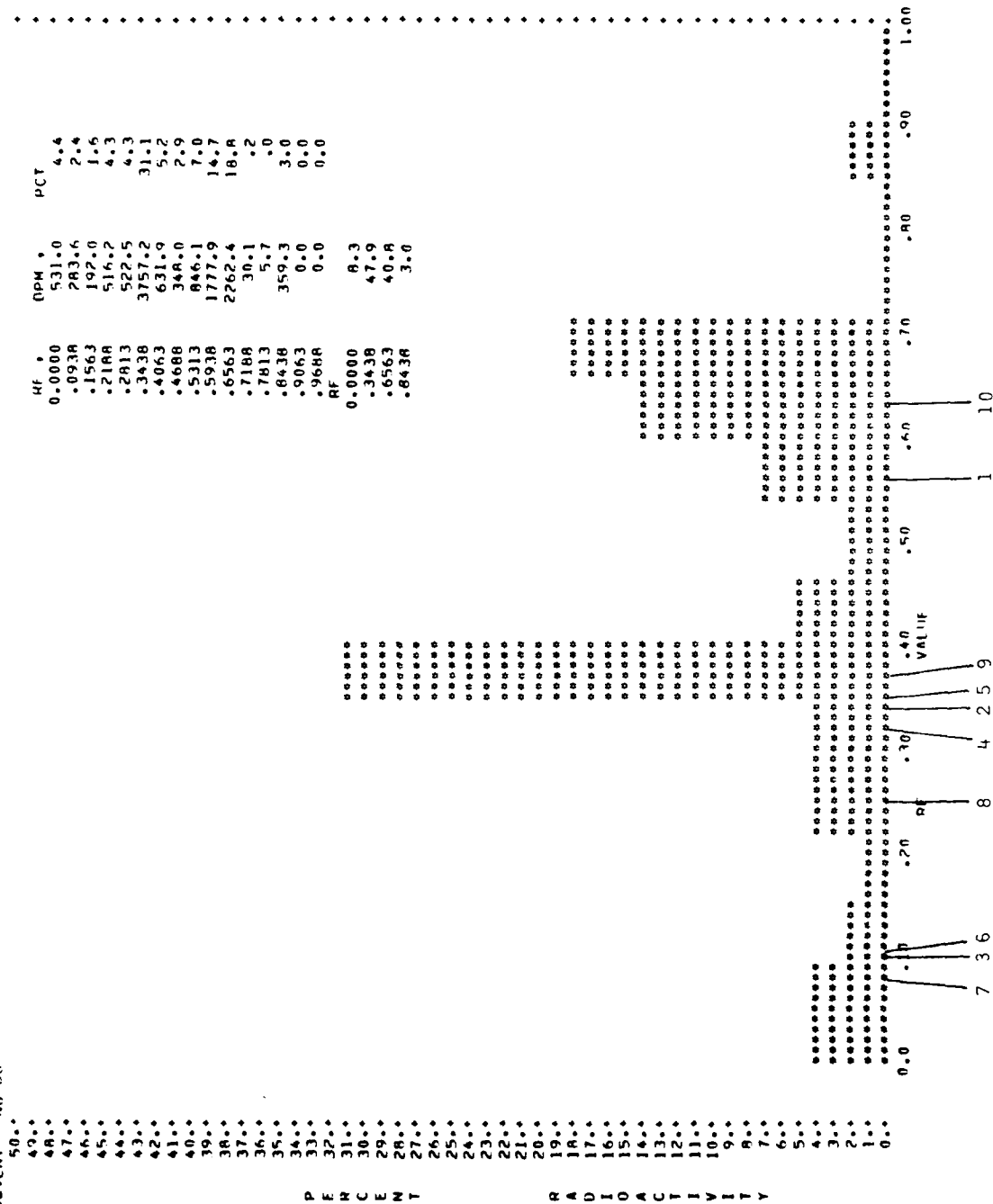


Figure 40-E6: Solvent IX

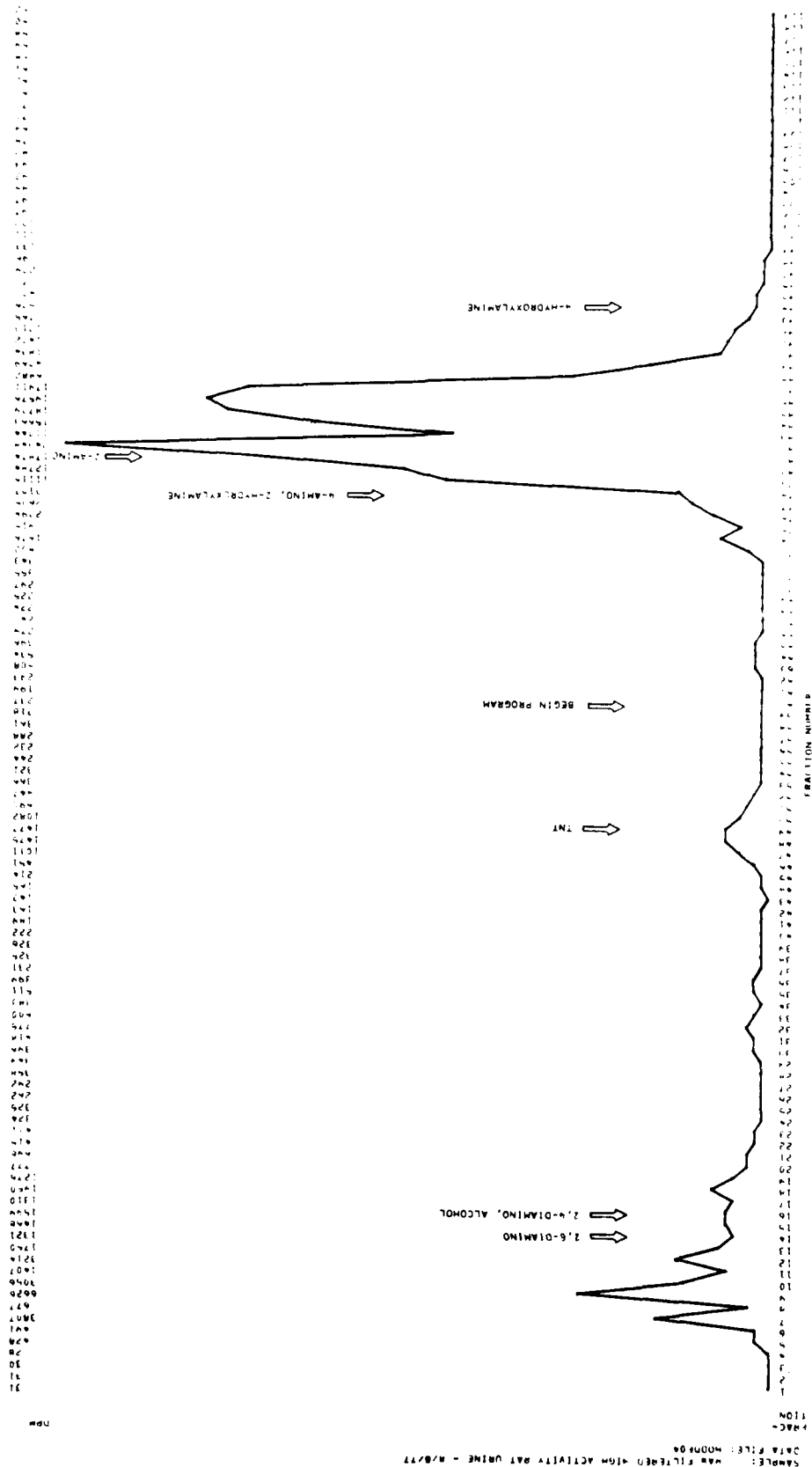


Figure 41 - HPLC of Rat Urine Obtained After Oral Administration of ^{14}C -TNT.
Volume of urine injected was 100 μl and 0.8 ml fractions were collected.

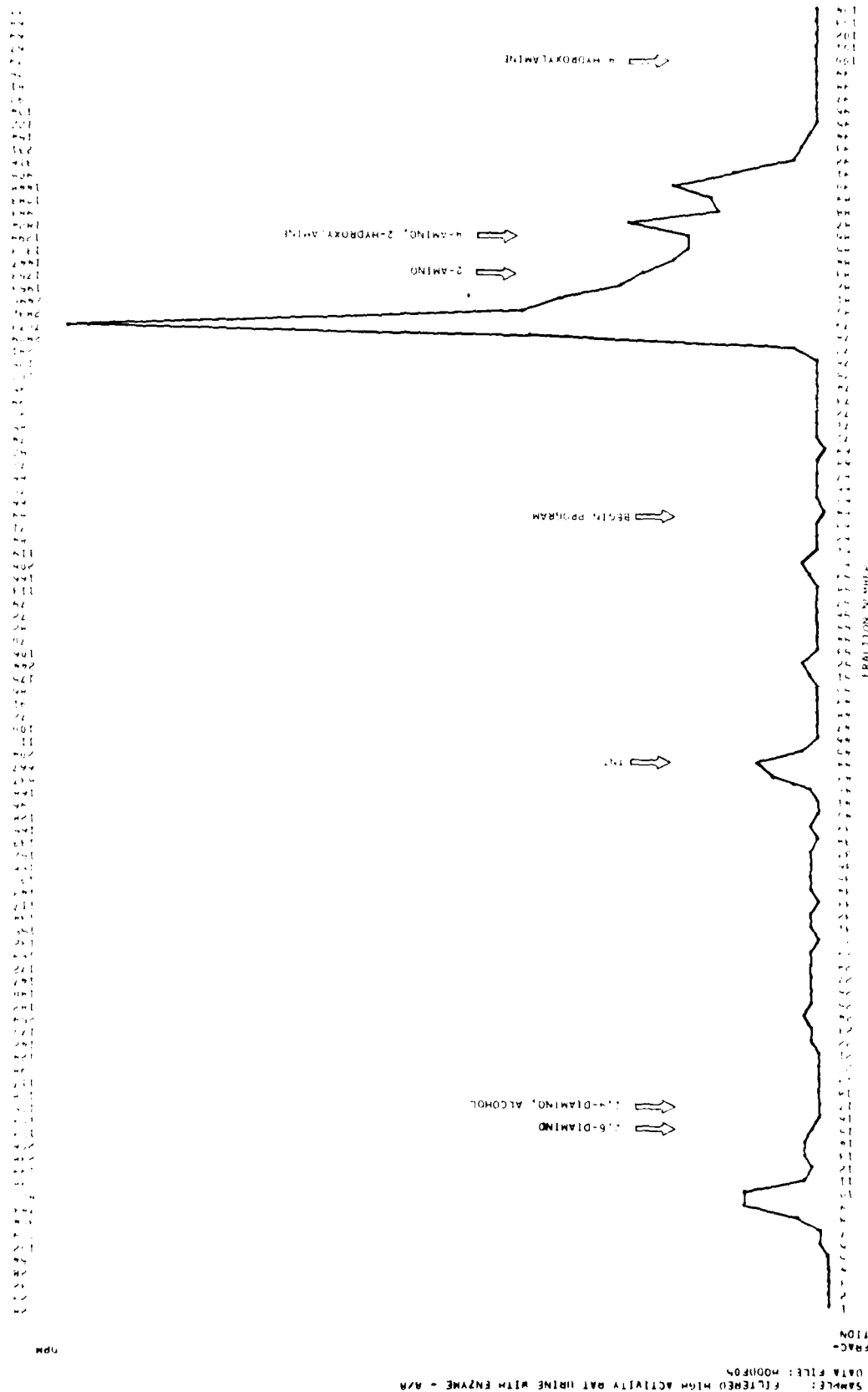


Figure 42 - HPLC of Rat Urine Obtained After Oral Administration of ^{14}C -TNT. Urine was treated with β -glucuronidase for 24 hr at 37° in the presence of acetate buffer (pH 5.0). Volume of urine injected was 25 μl and 0.8 ml fractions were collected.

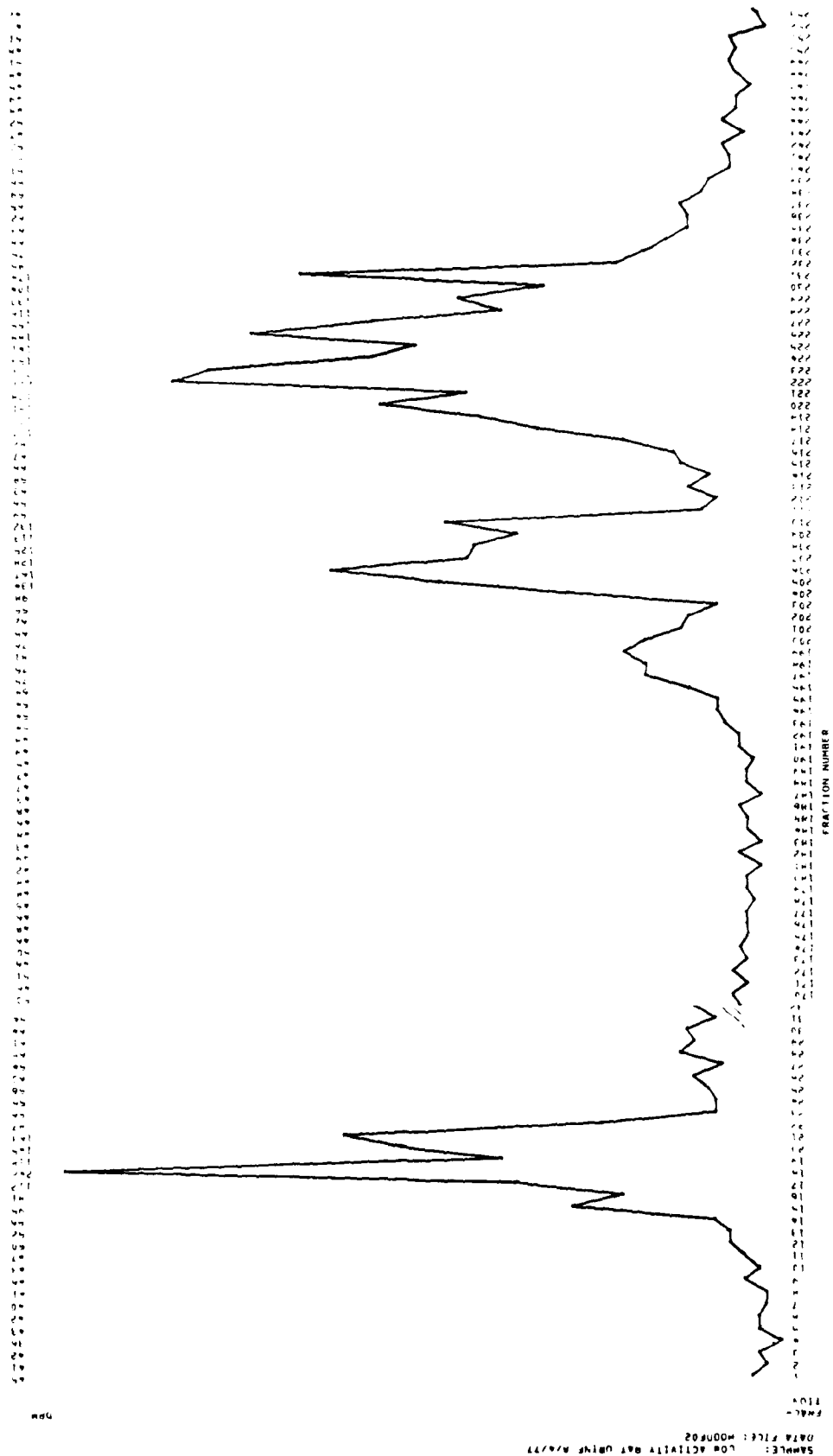


Figure 43 - HPLC of Rat Urine Obtained After Oral Administration of ^{14}C -TNT.
Volume of urine injected was 20 μl and 0.4 ml fractions were collected.

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